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A CONTRIBUTION TO THE FISHERY BIOLOGY OF A COMMON CYPRINID FISH, HEMIBARBUS BARBUS (T. ET S.)

Itsuo KUBO, Haruhiko UEMOTO and Kaichirō WATANABE

(Received Jan. 15, 1953)

No work has been carried out for the fishery biology of *Hemibarbus barbus* (T. et S.), an important fresh-water fish. We have made a study on this field of this animal, and have obtained some new findings important for the propagation of this fish as set forth later on. Headings concerned in this theme are as follows: Minimum size of the fish having scale; Season of annulus-formation; Relationship between lengths of scale and body; Body-length corresponding to each annulus; Weight-length relationship; and Body-length composition, age, and others of the spawning run.

Before proceeding any further we wish to express our hearty thanks to Mr. Jūichi HANARI and Messrs of the Station for the Promotion of Fishery of Ibaraki Prefecture for their assistance rendered in obtaining the material on which the present investigation is based. Also many thanks are extended to Professor Densaburō INABA and Mr. Morizumi NAKAMURA for their kindness offering young specimens to us, and to the Ministry of Education from which the present study has been given the grant in aid for foundamental scientific research.

(1) Material

The material used in the present investigation consists of 158 individuals, 1.6–45.0 cm in body-length, mostly taken by a kind of set net and a lift net with crossed bamboo supports from the Lake, Kasumiga-ura and the River Sakura-

Table 1. Date and localities on which the present specimens were taken, body-length (L), and others. A, Sotonasaka-ura, Ibaraki Pref.; B, Lake Biwa (mouth of the Inukami River); C, Kasumiga-ura (Kangaki); D, Sagami River (Atsugi); E, Tsuchi-ura (Sakuragawa).

Date	Localities	Collector	No. of Specimens	No. of Annuli	L (cm)
Dec. 23, 1947	Kihara	KUBO	11	0-3	10.0-25.4
Jan. 27, 1951	〃	〃	7	0-3	10.2-29.3
July 4, 〃	〃	〃	26	1-4	15.2-36.5
〃 7, 〃	A	INABA	3	0	3.2- 3.3
〃 26, 〃	B	NAKAMURA	4	0	1.6- 1.8
Aug. 3, 〃	C	INABA	2	0	4.7- 7.4
Sep. 30, 〃	Kihara	KUBO	5	0	7.9- 9.6
Nov. 6, 〃	〃	〃	53	0-5	7.4-40.5
〃 26, 〃	D	YAMANE	1	5	45.0
Apr. 12, 1952	E	KUBO	46	1-4	9.4-33.3

gawa, which flows into the lake in question, in months, January, April, July August, September, November, and December of the years, 1947, '51, and '52. Other details of this things are given in the Tables, 1–3. Moreover a set of data of body-length measured on 118 individuals, 5–34 cm long, by the Station for the Promotion of Fisheries of Ibaraki Prefecture on March 31 and April 7–8, 1952 is used.

For scale-analyses, five scales, from 13th to 17th, of the row just above the lateral line of the left side of the fish were taken, and the most suitable one among them is mounted for measurement because these scales are revealed to be subject to most regular in shape as given in the section of morphology of the scale.

Table 2. Number of specimens grouped by date, on which they were collected, and age (A_0, A_1, ..., A_5).

Date	No. of individuals of each age group						Total
	A_0	A_1	A_2	A_3	A_4	A_5	
Jan. 27	2		3	2			7
Apr. 12	1			32	13		46
July 4		2	7	16	1		26
July 7	3						3
July 26	4						4
Aug. 3	2						2
Sep. 30	5						5
Nov. 6	36	3		11	2	1	53
Nov. 26						1	1
Dec. 23	4	4	2	1			11
Total	57	9	12	62	16	2	158

Table 3. Number of specimens, range and average of body-length (L) in cm of each age-group.

Age	No. of Specimens	Range of L	Average of L
0	57	1.6-11.3	—
A_1	9	13.3-19.5	16.61
A_2	12	18.7-29.0	22.41
A_3	62	23.0-33.0	28.10
A_4	16	25.0-36.5	29.80
A_5	2	40.5-45.0	42.75

(2) Methods of body-length mensuration and scale reading, and notation of age

The body length used in this theme is referred to the standard length in mm, and represented by L.

The mensuration of scales was made under a magnification of 20 times by using a projector with the following procedures. The scales on which measurement has done, were projected by passing light as images on the frosted glass plate fixed on the projector. Along the longitudinal axis of the image a tracing paper of best quality is placed and the anterior and posterior edges of the

scale, focus, and the forerim of each annulus were marked off by a pencil on the ribbon. Of the ribbons with marks of those items given just above, measurements are rendered on such three matters as R, R', and r_1-r_n as illustrated in the Fig. 1. R is indicated by the distance from the focus to the anterior edge. R' is of distance from the focus to the posterior border of the scale. Each radius, r_1-r_n, is the length measured from the focus to the foremargin of each yearly ring.

Notation of age used in the present investigation is as follows :—A_0 represents that without annulus or yearly ring on the scale; A_1, A_2, ...A_5 express those having one, two, ...five yearly rings on the scale respectively. It may be noticed in this place that these symbols designate real age of this fish, because of the season of yearly ring formation of the scale is nearly in agreement with the spawning season of this animal as mentioned later on.

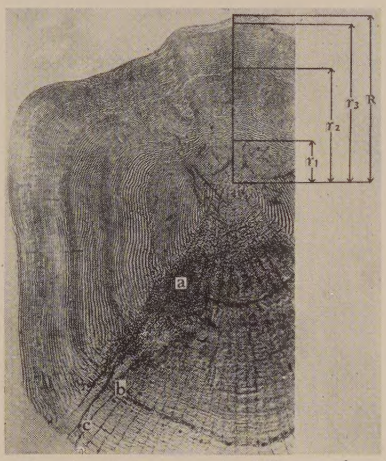

Fig. 1. Outer aspect of the acale of *Hemibarbus barbus* (T. et S.), and illustration of measurement. a–c, indicate the checks of the annuli found on the exposed area. R, R', r_1-r_3, see text.

(3) Morphology of the scale

The scales are cycloid, large, hexagonal in shape, 49 in number on the row of the lateral line. All elements useful for scale-analyses are well defined. So that reading of annuli is easy. The focus lies much nearer to anterior end than to posterior one of the scale. Annuli are characterized by possession of branched and broken circuli as shown in the Fig. 2, a and b. In addition to this each annulus shows the following character that the annulus continues to a chek running across the exposed area (Fig. 1, a–c). Also the circuli are found set closer together in posterior neighbouring area of annuli than in any other area. These characteristics afford important aids for recognition of the annuli.

To determine the scales which should be collected, an index is measured on all scales lying on the row just above the lateral line of a specimen, 37.9 cm in body-length. The index is calculated as W/S, where W indicates the width measured in the widest region near anterior shoulder of the scale, and S represents the length measured from the anterior rim to the posterior one. The value of the index ranges from 0.765 to 1.128 except that of regenerated scales, and shows maximum on the 8th scale, and minimum on the scales from 41st to 43rd. It is increasing rather rapidly from the 1st scale to the 8th one, nearly constant as being 1.020–1.035 on the scales from 12th to 17th, gradually decreasing on the scales from 18th to 33rd, rather abruptly diminishing on the scales from 35th to 40th, and apidly increases on the scales from 43rd to 47th as shown in the Fig. 3.

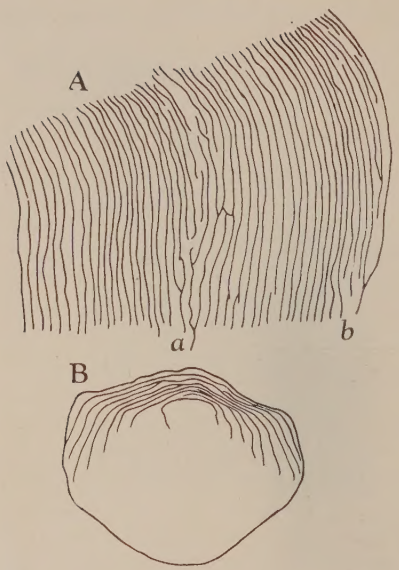

Fig. 2. A, a scale-part bearing annuli on middle (a) and outer edge (b), especially showing circuli of annuli basing on a scale taken from a female, 23 cm long fished on April 12, 1952, ×30; B, a scale removed from a specimen, 3.4 cm in body-length, ×30.

As seen above, the scales from 13th to 17th appear to be most regular in form. Further observations of this subject were made dealing with other four specimens, and similar results were obtained. Hence those 13th to 17th scales were used for the scale-analyses of the present inspection.

In the last place of this section, some descriptions of the scales removed from young specimens, 3.2–3.3 mm in body-length. The scales of those specimens are 0.65–0.87 mm in total length measured along the longitudinal axis, and 0.16 mm on average in *R*. Relationship between number of circuli and body-length, as well as scale-length are as follows. It seems to be that the number rapidly increases until the scale-length (*R*) reaches to about 0.55 mm. The circuli ranges from 7 to 9 in number in the scales of 0.65 mm in length, and from 12 to 15 in those of 0.87 mm long.

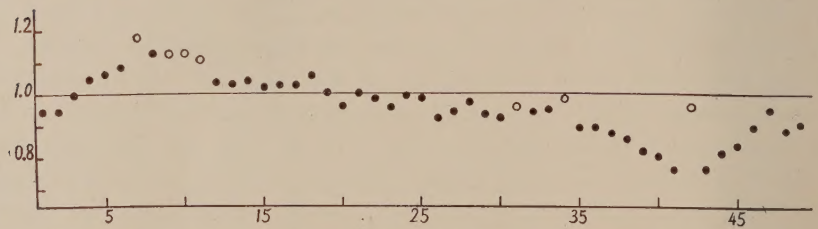

Fig. 3. The index, *W*/*S* (see text) in relation to scales from 1st to 49th (Horizontal axis). White circles, regenerated scales.

(4) Minimum size of the fish with scale

The body-length of the fish when the scales were first laid down is unknown. To reveal this matter, seven youngs, 1.6–3.3 cm in body-length, were examined. Among them, four ones measuring 1.6–1.8 cm in body-length taken from the Lake Biwa on July 26, 1951 were found without scales on whole body-surface. But those with body-lengths 3.2–3.3 cm caught from Sotonasakaura, Ibaraki Prefecture on July 7, 1951 were observed already provided with scales of

many sizes as set forth already in the section of morphology of the scale.

On the basis given above, it may be assumed that the scales of this fish firstly occur when the body-length measures about 2.5 cm. This value is well in accordance with that obtained by extrapolation of the regression line of $R-L$ logarithmic diagram (Fig. 5). But this value is a little greater than those of Japanese carp, *Cyprinus carpio* L. and Squawfish, *Ptychocheilus grandis*. It is 1.6–1.7 cm in the former according to NOZAWA's careful investigation,[3] andab out 2.0 cm in the latter[5] by the study of TAFT and MURPHY.

(5) Season of annulus-formation

For the purpose to know the time of annulus-formation, width of the "Terminal zone"[4] were measured, and an index ($R-r_n/R$, where r_n indicates the length from the focus to the outer edge of the last annulus), known hereafter as terminal index, is calculated on all specimens available for this purpose. As shown in Fig. 4, the index shows some extent of variation within even the same year-class, and they appear to be greatly different by age. But it may be said that the indexes of younger age-classes are larger than those of older ones in

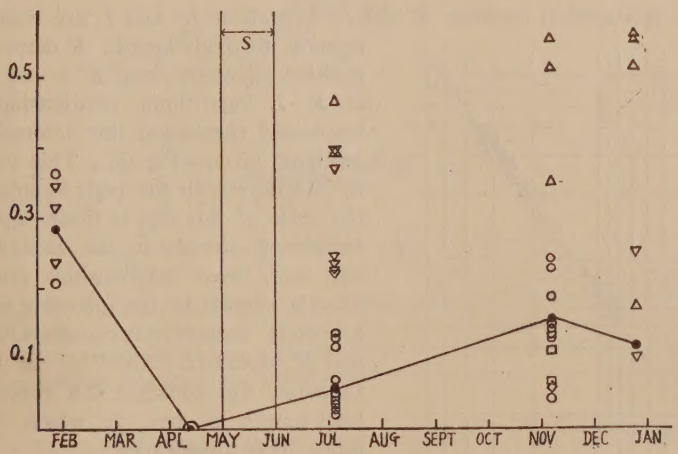

Fig. 4. Terminal indexes of scales of *H. barbus*. Triangles, 1-age (A_1); Inverted triangles, 2-age (A_2); White circles, 3-age (A_3); Black ones, averages of the same age-class; Squares, 4-age (A_4); Rhombs, 5-age (A_5). *S*, spawning period.

months considerably remote from the season of annulus formation. This difference may be ascribed to the difference of growth rate of those age-classes. The material dealt with in this investigation unfortunately lacks one- and two-annuli specimens of the seasons from early Spring to early Summer. Therefore analysis of this item is based hereafter on 3-annuli fishes only. Ranges and averages of the index of 3-annuli group are given in the Table 4. According to the Table 4 and Fig. 4, the minimum of the index is found to exist on the scales of the specimens caught on April 12. Judging from such things given above, it appears

Table 4. Terminal index of 3-annuli group.

Date on which the specimens were taken Month	Day	Number of specimens	Terminal index Range	Averrge
1	27	2	0.2075-0.3625	0.285
4	12	32		0
7	4	16	0.0200-0.1370	0.058
11	6	10	0.0425-0.2425	0.157
12	23	1		0.117

to be that the annulus-formation of scale of this fish takes place during spring time of months, March and April. Then, the spawning season of this cyprinid is generally known as being early Summer from May to June (pp. 158–159).[3] Wherefore it may be said that the first annulus developed on the scale indicates one age of full year. Accordingly the annuli of the scale may be thought to show the age of the animal in complete year.

(6) Relationship between lengths of scale and body

The relationship between R and L, as well as R' and L are examined. In relation to body-length, R demonstrates a positive allometry, and R' a negative one. In $R-L$ logarithmic relationship, the extrapolated regression line intersects L-axis at about 2.5 cm (Fig. 5). This value iswell in harmony with the body-length, in which the scale of this fish is firstly appeared as mentioned already in the Section (4). At any rate, those relationships given above may be shown by the following exponential functions respectively: $R=0.0289\ L^{0.13733}$ and $R'=0.424\ (L-2.5)^{0.816}$. In the latter function, the constant 2.5 represents the body-length in cm, at which the scales made their appearance.

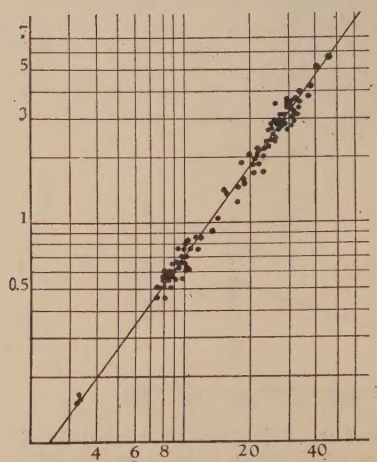

Fig. 5. Showing relationship between scale-length (R, in mm) and standard body-length in cm on double logarithmic scale. Horizontal axis, body-length. The regression line fitted by the least square method.

(7) Position of each annulus from focus, and body-length corresponding to each annulus

The value of each r shows some extent of variation, and there is a tendency that the variation becomes gradually larger with advance of the order of r. But the frequency distribution of each group of r_1-r_3 has been proved to be well defined each other, though the groups of r_3 and r_4 are not well defined as shown in the Figs. 6 and 7. The mean (M), σ, and $M\pm3\sigma$ are given in the Table 5.

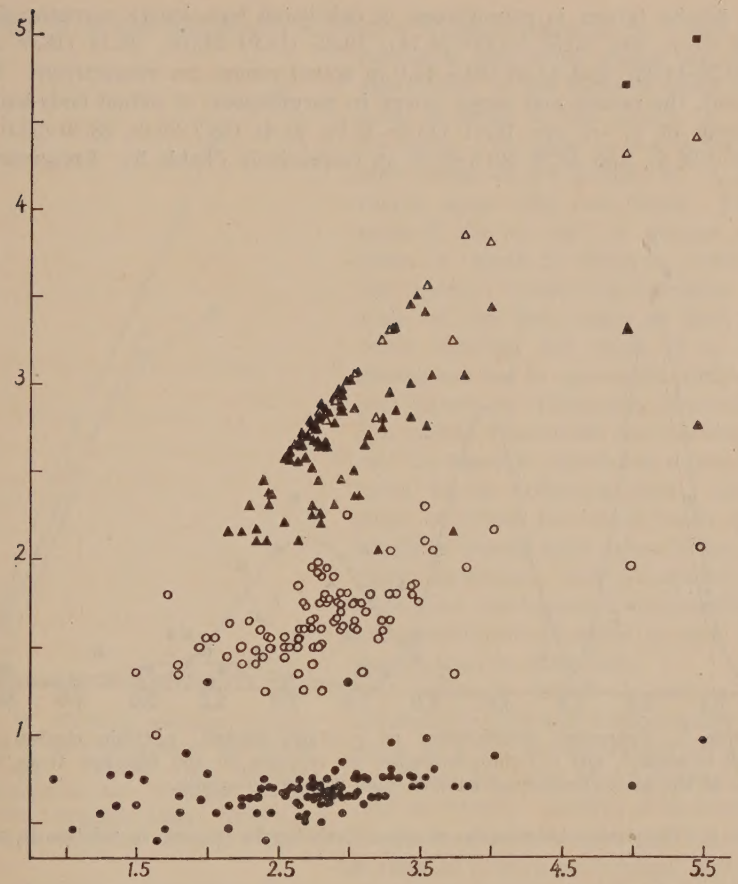

Fig. 6. Showing location of annuli of all scale-samples examined. Horizontal axis, scale-length (R) in mm; Vertical one, distance from focus of scale to outer edge of each annulus in mm. Black circles, r_1; White circles, r_2; Black triangles, r_3; White triangles, r_4; Black squares, r_5.

Table 5. Average (M) and standard deviation (σ) of r_1-r_5 in mm, number of specimens dealt (No.), calculated (L') and actual (L) body-lengths in cm.

Items	r_1	r_2	r_3	r_4	r_5
No.	9	12	62	16	2
r (M)	0·688	1.659	2.682	3.031	4.825
r (σ)	0.137	0.230	0.358	0.775	—
r ($M\pm3\sigma$)	0.277~1.099	0.969~2.349	1.608~3.756	0.706~5.356	—
L' (M)	10.58	19.35	27.14	29.60	41.50
L' ($M\pm3\sigma$)	5.19 ~14.15	12.91~24.59	18.66~34.61	10.25~44.82	—
L	16.61	22.41	28.10	29.80	42.75

M and $M\pm3\sigma$ (given in parentheses) of calculated body-length corresponding to each of $r_1\sim r_5$ are 10.58 (5.19~14.14), 19.35 (12.91–24.59), 27.14 (18.66–34.61), 29.60 (10.25–44.82), and 41.50 (40.5–45.0 in actual range) cm respectively. In the other hand, the means and range (given in parentheses) of actual body-length of each group of r_1-r_5 are 16.61 (13.3~19.5), 22.41 (18.7–29.0), 28.10 (23.0–33.0), 29.80 (25.0–36.5), and 42.75 (40.5–45.0) cm respectively (Table 5). Frequency dis-

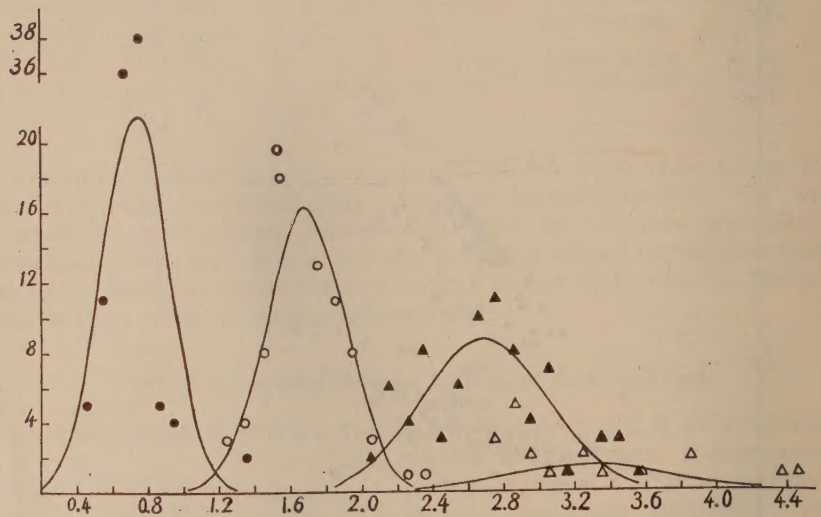

Fig. 7. Frequency distributions of r_1 (black circles), r_2 (white circles), r_3 (black triangles), and r_4 (white triangles) in relation to the distance from the focus of the scale (Horizontal axis) in each 0.1 mm grouping.

Table 6. Frequency distribution of actual body-length (L, cm) in relation to age (A_0-A_5).

L \ Age	A_0	A_1	A_2	A_3	A_4	A_5	Total
0- 3	4						4
3- 6	4						4
6- 9	23						23
9-12	26						26
12-15		2					2
15-18		4					4
18-21		3	4				7
21-24			5	2			7
24-27			2	16	1		19
27-30			1	25	8		34
30-33				17	5		22
33-36				2			2
36-39					2		2
39-42						1	1
42-45							0
45-48						1	1
Total	57	9	12	62	16	2	158

tribution of actual body-length in relation to age is given in the Table 6. Both calculated and actual means should be in agreement, if the latter is based on specimens secured in the season of annulus-formation. As seen in the Table 5, those both means of the groups, r_3, r_4 and r_5, nearly agree with each other. The specimens of the r_3- and r_4- groups are that taken in April, in which or a little before this month, yearly-ring-formation of the scale of this fish seems to take place as noted already. But those of r_1- and r_2- groups are not in agreement with considerable extent of differences between them. The causes responsible for this discrepancy may be, however, attributed to the respects given in the following lines. The specimens, on which the actual mean of body-length is based, were taken in the months (July, November, and December) far remote from the season of annulus-formation, and growth is much faster in such younger groups than in older ones.

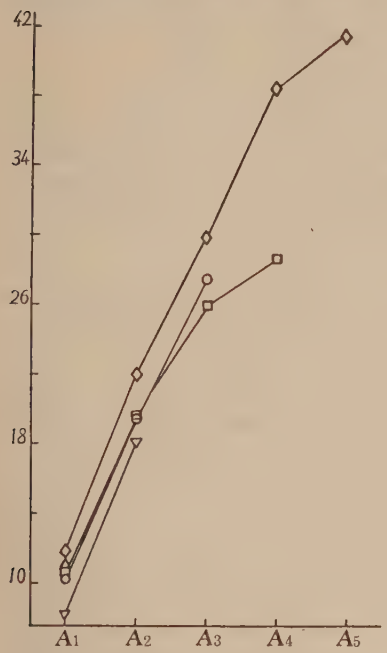

Fig. 8. Showing average growth-curves of each age-group. Horizontal axis, age; Vertical one, standard body-length in cm. Triangle, 1-age; Inverted triangles, 2-age; Circles, 3-age; Squares, 4-age: Rhombs, 5-age.

Average values of each r_1—r_5 and calculated body-length corresponding to each value of r_1—r_5 are mentioned on the Table 7. From this table, the following matters may be known. Growth of each age-group is not equal as shown in the Fig. 8. But it runs as follows on average, namely, the fish measure 10.4, 19.8, 27.6, 33.5, and 41.5 cm in body-length at the time of completion of yearly-ring in A_1, A_2, ..., A_5 respectively. It follows therefore that the body-length of A_2, A_3, A_4, and A_5 is about 1.9, 2.7, 3.2, and 4.0 times to that of A_1 respectively. Growth increment is about 90% between A_1 and A_2, cir. 40% between A_2 and A_3, about 20% in both cases between A_3 and A_4, and A_4 and A_5.

Table 7. Average values of each r_1-r_5, calculated (given in parentheses) and actual (L) body-lengths in cm.

Age	r_1	r_2	r_3	r_4	r_5	L
A_1	0.76(11.0)					16.61
A_2	0.59(8.2)	1.51(18.0)				22.41
A_3	0.68(10.2)	1.68(19.4)	2.71(27.4)			28.10
A_4	0.72(10.6)	1.69(19.6)	2.52(25.9)	2.86(28.6)		29.80
A_5	0.83(11.8)	2.00(22.0)	3.03(29.8)	4.35(38.4)	4.83(41.5)	42.75
Average	0.71(10.4)	1.72(19.8)	2.75(2.76)	3.60(33.5)	4.83(41.5)	

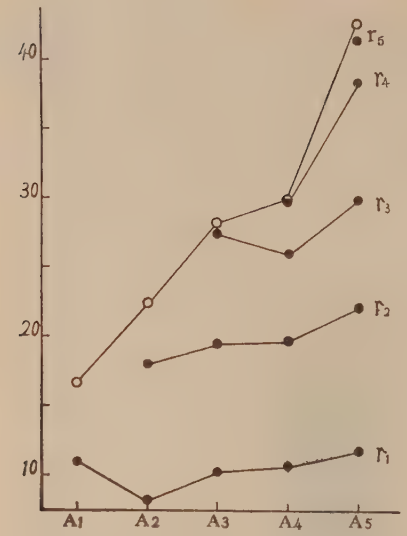

Fig. 9. Showing average actual body-lengths (white circles) and calculated body-lengths (black circles) corresponding to each r. Abscissa, age; Ordinate, body-length in cm.

Finally it should be mentioned herewith that an anti-Lee-phenomenon, rather evident, is present as seen in the Table 7 and Fig. 9. Percentages of r of all age-groups against mean of r of each age-group (Table 8) and those of r of all age-groups to r of all ones as exhibited on the Table 9. For correction of body-length calculated by use of scale, those Tables given above are available.

(8) Weight-length relationship

The relationship between body-length (L) and body-weight (W) is examined on all available specimens. No sexual difference is found to exist, at least, within the limit of the material dealt with. The relation is of straight line in regression on a double logalithmic scale as shown in the Fig. 10. The regression line, fitted by means of the least square method, may be represented by the following power law : $W=0.0173\ L^{3.0005}$. The exponent closely approximates to 3.

Table 8. Percentages of r of all age-groups against mean of r of each age-group. ar_1, shows r_1 of age A_1; br_1, means r_1 of age A_2; br_2, shows r_2 of age A_2; cr_2, shows r_2 of age A_3; and so on. mr_1, shows mean of r_1 of all age-groups; mr_2, mean of r_2 of all age-groups; and so on.

ar_1/mr_1	1.05							
br_1/mr_1	0.73	br_2/mr_2	0.90					
cr_1/mr_1	0.98	cr_2/mr_2	0.97	cr_3/mr_3	0.99			
dr_1/mr_1	1.01	dr_2/mr_2	0.98	dr_3/mr_3	0.93	dr_4/mr_4	0.85	
er_1/mr_1	1.13	er_2/mr_2	1.11	er_3/mr_3	1.07	er_4/mr_4	1.14	er_5/mr_5

Table 9. Percentages of r of all age-groups against r of all ones. Symbols used are the same as is the case with the table 8.

ar_1/er_1	93.22						
br_1/er_1	69.49	br_2/er_2	81.81				
cr_1/er_1	86.44	cr_2/er_2	88.18	cr_3/er_3	91.94		
dr_1/er_1	89.83	dr_2/er_2	89.09	dr_3/er_3	86.91	dr_4/er_4	77.08
ar_1/dr_1	103.77						
br_1/dr_1	77.35	br_2/dr_2	91.83				
cr_1/dr_1	96.22	cr_2/dr_2	98.97	cr_3/dr_3	105.79		
ar_1/cr_1	107.84						
br_1/cr_1	80.39	br_2/cr_2	92.78				
ar_1/br_1	134.14						

(9) Body-length composition, age, and others of the spawning run

In the Lake, Kasumiga-ura, the fish in question run up in spawning season to the rivers which empty into the Lake. The Ibaraki Prefectural Station for Promotion of Fishery has measured 71 individuals belonging to this spawning run and fished by a lift net with crossed bamboo supports at a locality about 2 km from the mouth of the River, Sakuragawa, on March 31 and April 25, 1952. The senior author of the present paper has also measured 43 fish caught by the same fishing gear at the same locality on April 12, 1952. Body-length composition basing on them is given on the Fig. 11. It seems that, at least, two groups are present to exist. The fishes comprised in the smaller sized group belong to ages of A_0 and A_1 having body-length less than 13 cm and body-weight less than 17.0 g. Those of the larger sized one appears to be mostly in ages of A_3 and A_4. Owing to the material composed of 43 specimens obtained on April 12, 1952, the body-length ranges 24.0–33.3 cm (27.6 cm on average) in fish of age A_3, and 26.5–32.5 cm (29.12 cm on average) in those of age A_4. The body-weight runs 225.0~637.5 (379.3 g on average) in the fish of age A_3, and 281.2–562.5 (403.9 g on average) in the fish of age A_4. Of those 43 specimens, only 2 (4.6 %) are in age of A_2, 30 (69.8%) are in age of A_3, and the rest (25.6%) are in age of A_4. Accordingly it may be said that the spawning run of this fish consists of 3- and 4- age groups, mostly of the former.

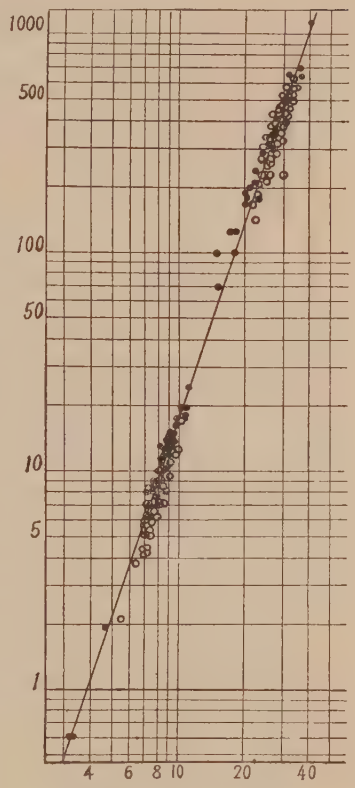

Fig. 10. Weight-length relationship of *H. barbus* on a double logarithmic scale. Horizontal axis, body-length in cm; Vertical one, body-weight in *g*. The regression line is fitted by the least square method. White circles are based on samples taken from spawning run.

Among those 41 specimens of age A_3 and A_4, 15 (36.58%) are male, and the rest (63.42%) are female as the sex is determined in all cases by presence or absence of pearl organs on head and other bodily parts. The difference between the observed sex-ratio and the theoretically expected sex-ratio of 1 may be examined by the chi-square test. χ^2 and P-values were observed as being 1.010 and $P>0.30$ respectively. It may be concluded, therefore, that the observed ratio of males to females for the total samples taken on April 12 does not differ significantly from the theoretically expected ratio.

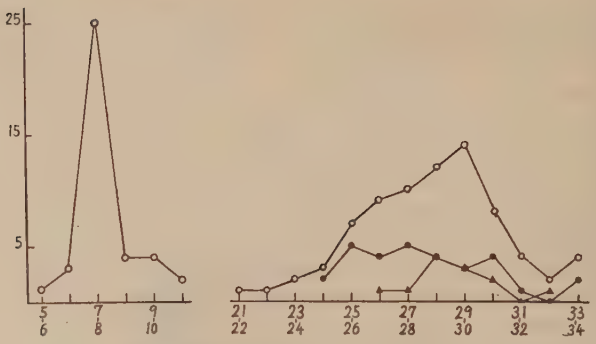

Fig. 11. Size-frequencies of spawning run, 114 individuals in total, fished from the River, Sakuragawa, on March 31, April 12, and 25 (white circles). Horizontal axis, body-length in cm. Black circles, 3-age fish caught on April 12; Black triangles, 4-age one obtained on the same date.

(10) Summary

(1) A fishery biological study of a Japanese Cyprinid fish, *Hemibarbus barbus* (T. et S.) has made basing on a material comprising 158 specimens, 1.6–45.0 cm in standard length, taken mostly from the Lake, Kasumiga-ura.

(2) The scale of this fish is revealed to be useful for age-determination as it is very legible.

(3) The annuli are characterized having branched and broken circuli on the covered area, and are found as a check on the exposed area.

(4) The minimum size bearing scales is about 2.5 cm in standard body-length.

(5) Annulus-formation of scale appears to take place during spring time of such months, March and April basing on an analysis of the terminal index.

(6) Regarding scale, the length (R) from the focus to the anterior margin shows a positive allometry, and that (R') from the focus to the posterior border demonstrates a negative allometry in relation to bydy-length (L) respectively. The relationship between R and L may be given by the following exponential functions: $R=0.0289\ L^{1.3733}$ and $R'=0.424\ (L-2.5)^{0.816}$, the constant 2.5 represents the minimum size with scales in body-length in cm.

(7) Calculated body-lengths corresponding to r_1, r_2, ..., r_5 are 10.58, 19.35, 27.14, 29.60, and 41.50 cm respectively.

(8) The growth of each age-class of this fish may be run as follows designating it by calculated body-length, viz., 11.0 cm at r_1 of A_1; 8.2 cm at r_1 and 18.0 cm at r_2 of A_2; 10.2 cm at r_1, 19.4 cm at r_2, and 27.4 cm at r_3 of A_3; 10.6 cm at r_1, 19.6 cm at r_2, 25.9 cm at r_3, 28.6 cm at r_4 of A_4; and 11.8 cm at r_1, 22.0 cm at r_2, 29.8 cm at r_3, 38.4 cm at r_4, and 41.5 cm at r_5 of A_5.

(9) A remarkable anti-Lee-phenomenon is found to exist.
(10) The body-weight (W) increases with the body-length (L) at a rate of nearly cube as expressed by the following formula irrespective of sex: $W=0.0173\ L^{3.0005}$.
(11) The spawning run of this fish appears to be composed of 3- and 4-age groups, mostly of the former.
(12) The sex ratio of the spawning run seems to be 1.

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A SCALE-ANALYSIS ON *SEBASTOLOBUS MACROCHIR*, WITH SPECIAL REFERENCE TO THE AGE-DETERMINATION

Jin Hattori

(Received Feb. 10, 1953)

Sebastolobus macrochir (Günther) is one of the fish important as a resource for the Japanese bottom trawl and long line fisheries, since the fish appears in considerable abundance in the waters on the Pacific coast of Hokkaidō and Northeastern part of Japan. An age-determination of this fish, of which little contribution has been done so far as my knowledge is concerned, has been attempted by means of scale analysis.

Before proceeding any further, I take this opportunity of expressing my hearty thanks to Professor Dr. Itsuo Kubo of our university under whose direction and encouragement the present study was initiated and accomplished, and by whose suggestions, corrections have been made in preparing this manuscript. Acknowledgement is made to Professor Dr. Hiroshi Niino, belonging to the university, by whom the chance of sampling were afforded, and also to Messrs. Kazunori Takagi of the same university and Zirō Kinoe, of the Tōhoku Sea Regional Fisheries Research Laboratory, for their help in various ways.

(1) Material and method

The material consists of 266 individuals ranging 4.5–27.0 cm in body-length. One set of material was caught off the Cape Erimo, Hokkaidō on August 4, 5, 1951 by the Seichōmaru, the training ship of our university, by "menuke" long line. The measurement of body-length and sampling of scales on which the present work is based were made on board of the ship. Other one was fished off the Cape Erimo on December 3, 1951, off Chōshi, Chiba Pref. on February 15, 1952, and off Hachinohe, Aomori Pref. on February 19, 1952 by coastal trawlers. In this case, measurement and sampling of scales were undertaken on specimens fixed by about 10% formalin solution commercially sold (Table 1).

Table 1. Collections used in the present study.

Locality	Date			Number of fish treated	Number of scales used	Range of body-length (cm)	Mood of body-length (cm)
Erimo	1951	VIII	4	100	65	14–26	23
Erimo	〃	〃	6	92	82	19–27	24
Erimo	〃	XII	3	16	16	8–15	12
Chōshi	1952	II	15	36	36	4–9	8
Hachinohe	〃	〃	19	22	20	8–21	12
Total				266	219		

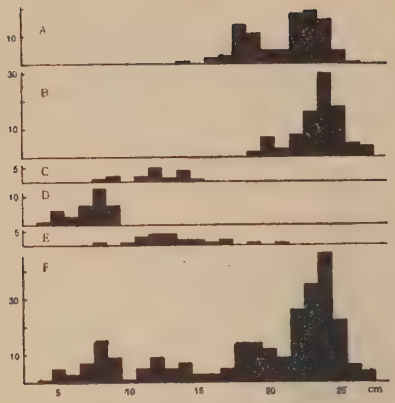

Fig. 1. Body-length composition of the present material. Ordinate, frequencies; Abcissa, body-length in cm; A, based on 100 individuals secured off the Cape Erimo on Aug. 4, 1951; B, based on 92 ones taken off the Cape Erimo on Aug. 5, 1951; C, based 16 ones obtained off the Cape Erimo on Dec. 3, 1951; D, based 36 ones caught off the Chōshi on Feb. 15, 1952; E, based 22 ones fished off the Hachinohe on Feb. 19, 1952; F, based 266 ones, total of the present material.

The body-length is given as a length covering from the tip of snout to the base of caudal fin. Its composition is given in the Fig. 1.

A number of scales was taken from vicinity of the region near the lateral line and covered by the tip of pectoral fin of individual specimen. A scale giving most legible picture and regular form was chosen among those obtained from each specimen dealt with for further examination. Then, on the whole, 219 scales useful for reading came under my examination from all the specimens, 266 in number. Since some specimens are without scales, and some ones with regenerated scales. For reading of resting zones and measurements, all available scales were mounted on slide glass with a drop of water after cleaning off adherent matters on them. They are observed under a projecter by passing light at an enlargement of 20 times. Measurements have made on the following two items: S, $r_1 \ldots r_n$ (Fig. 2, A). S represents the scale-length from the focus to the anterior edge of the scale, $r_1 \ldots r_n$ are showing the distance between the focus and the anterior rim of each resting zone along the median longitudinal axis.

The resting zones of the scale of this fish are made up of a small number of closely set circuli. Accordingly the recognition of them is rather easy. But the reading of the yearly rings of the scale is no easy task, because of the scale gives very complex picture having many false. So that I read out recognizable resting zones irrespective of degree of distinction of those zones to avoid personal error of reading as far as can be done (Fig. 2, B, C).

(2) Relationship between scale-length and body-length

Within the limit of the samples at my disposal, the maximum of scale-length is 8.70 mm and the minimum of it is 0.75 mm (corresponding body-lengths 26.0 cm and 4.50 cm respectively). The relationship between the scale-length (S) and the body-length (L) appears to show a trend of an exponential function as given in the Fig. 3. The regression line may be represented by the following formula, $S=0.410(L-C)^{0.907}$. The two parameters of this formula were obtained basing on the least square method. The constant C means the body-length at which the scale firstly developed. But the value is not yet unknown, since the material available for determination of it unfortunately is not

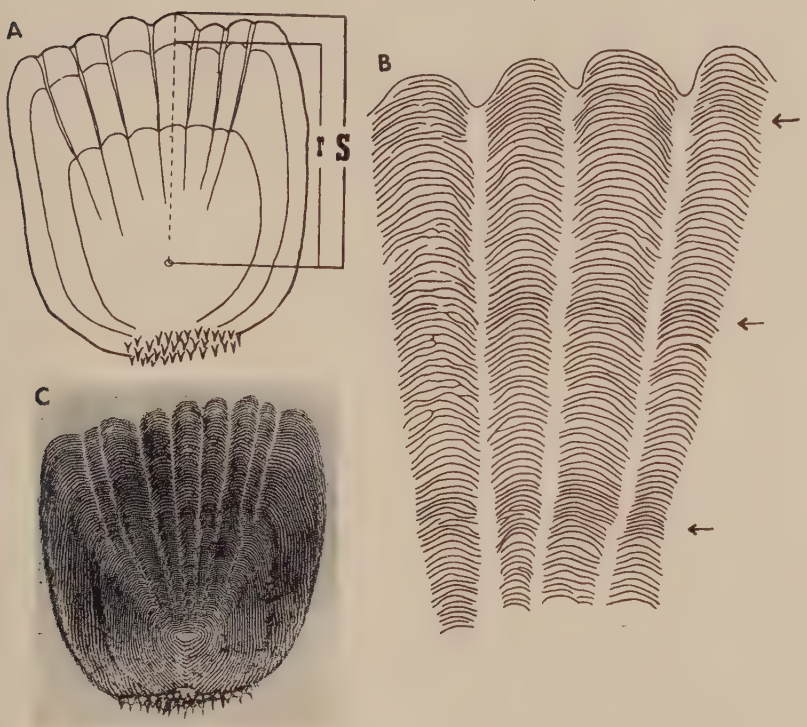

Fig. 2. Showing scale characters. A, explanation of measuring method of scale; B, details of scale especially showing resting zones (pointed by arrows) ×20; C, photograph of a scale of a young, 9.0 cm long, ×8.

obtained in the present investigation. It was estimated, therefore, by the following method. Then the C was determined basing on the Freser's formula, $\frac{L-C}{L_n-C}=\frac{S}{S_n}$, fitting actual body-length and scale-length of two specimens different in size. The C was calculated as being 2.3 cm on average of five times. This value approximates to the body-length at the time of the scale firstly appeared of *Sebastes marinus*,[1] which is an Atlantic form and affined to the present fish.

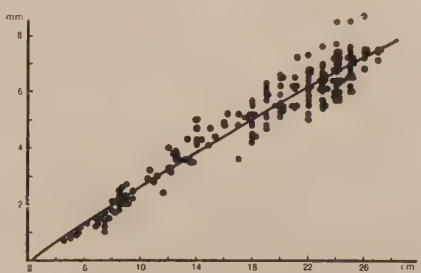

Fig. 3. Showing relationship between body-length in cm and scale-length in mm. Abscissa, body-length; Ordinate, scale-length.

(3) The time of resting zone formation

To discover the time of the resting zone formation of scale of the fish, two indexes, viz. $\frac{r_n-r_{n-1}}{r_n}$ and $\frac{r_{n-1}-r_{n-2}}{r_{n-1}}$, were used in addition to the terminal index, $\frac{S-r_n}{S}$. The reason why two other indexes was employed for this purpose in this place is that the material dealt with in the present investigation is not that collected continuously.

As seen in the Table 2 and Fig. 4, the indexes mentioned above are subject to considerable variation. But it may be noticed herewith that the indexes, viz., $\frac{r_n-r_{n-1}}{r_n}$ (Fig. 4, B), and $\frac{r_{n-1}-r_{n-2}}{r_{n-1}}$ (Fig. 4, C) of the August material, as well as $\frac{r_n-r_{n-1}}{r_n}$ (Fig. 4, E) of the February one, are similar in frequency distribution of modal part. Also the same condition is seen in the indexes, namely $\frac{S-r_n}{S}$ (Fig. 4, A) of the August material and that (Fig. 4, D) of the February one, though the values are much smaller than those of $\frac{r_n-r_{n-1}}{r_n}$ and $\frac{r_{n-1}-r_{n-2}}{r_{n-1}}$ (Table 2). Judging from the matter given above, it may be said that r_n-r_{n-1} and $r_{n-1}-r_{n-2}$ are equal in growth increment. So that if the material dealt with has been taken at the time the annuli completely achieved, both the indexes, $\frac{S-r_n}{S}$ and $\frac{r_n-r_{n-1}}{r_n}$ or $\frac{r_{n-1}-r_{n-2}}{r_{n-1}}$, should be the same or nearly so. While in the present material, the indexes of $\frac{S-r_n}{S}$ of Erimo, Chōshi and Hachinohe materials caught in August and February are considerably smaller than those of $\frac{r_n-r_{n-1}}{r_n}$ or $\frac{r_{n-1}-r_{n-2}}{r_{n-1}}$ as being about half of the latter two ones shown in the Table 2. And the indexes of the materials of August and February are nearly equal in values. Therefore it may be considered that

Table 2. Indexes used to infer the time of resting zone formation.

Index	Date	Locality	Range of mordal part	Average and S.D.
$\frac{S-r_n}{S}$	Aug.	Erimo	0.01—0.22	0.10±0.043
〃	Feb.	Chōshi, Hachinohe	〃	0.14±0.051
$\frac{r_n-r_{n-1}}{r_n}$	Aug.	Erimo	0.07—0.37	0.21±0.047
〃	Feb.	Chōshi, Hachinohe	〃	0.23±0.037
$\frac{r_{n-1}-r_{n-2}}{r_{n-1}}$	Aug.	Erimo	〃	0.22±0.043

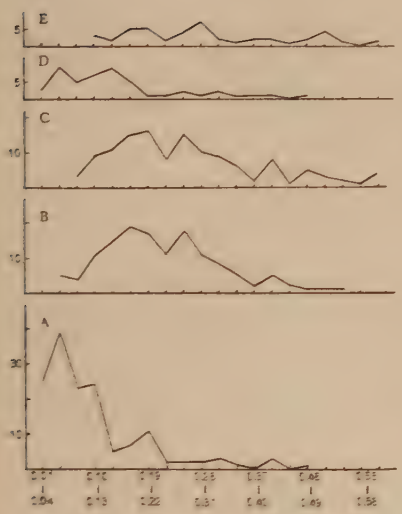

Fig. 4. Frequency distributions of the terminal index and other indexes. Abscissa, index. $\frac{s-r_n}{s}$, $\frac{r_n-r_{n-1}}{r_n}$, $\frac{r_{n-1}-r_{n-2}}{r_{n-1}}$ basing on the material taken from the waters Erimo in Aug., 1951 are given in A, B, C respectively. $\frac{s-r_n}{s}$, $\frac{r_n-r_{n-1}}{r_n}$ basing on the material taken from the waters Chōshi, Hachinohe in Feb., 1952 are given in D, E respectively.

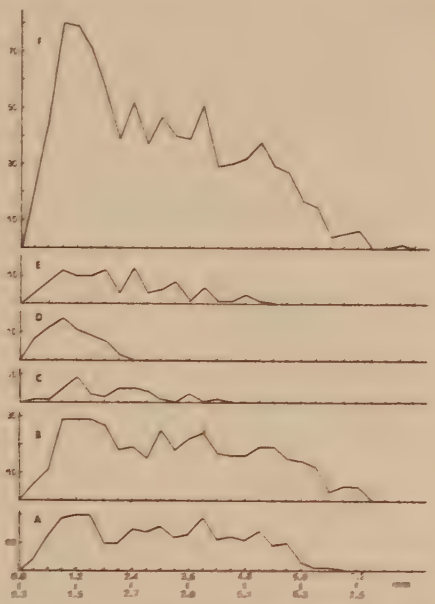

Fig. 5. Frequency distribution of the resting zones assorted by 0.3 mm of the distance from the focus (Horizontal Axis). A, Erimo specimens taken on Aug. 4; B, Erimo specimens taken on Aug. 6; C, Erimo specimens taken on Dec. 3; D. Chōshi specimens taken on Feb. 15; E, Hachinohe specimens taken on Feb. 19; F, Total of A-E.

there is two cycles of resting zone formation, and it may be assumed that the resting zone formation takes place in the months of May and June, and in those of November and December.

(4) Position of resting zone in scale

The resting zones laid down on the scale are commonly believed to be formed at similary position in the fish of unique stock, although it is inevitable that there is some individual variations. Accordingly the frequency distribution of the position of the resting zones should yields some peaks in the case plotted in relation to the distance measured from the focus of the scale. At analysis of this subject, several grouping intervals of the position, namely 0.1, 0.2, 0.3, 0.4 and 0.5 mm. were examined, and it was revealed that the 0.3 mm interval appears to give most suitable result.

The frequency distributions of resting zones in relation to their position are given in the Fig. 5. In regard to this item, it seems to be that less local

defference is present. Because of each curve, shown on the Fig. 5, A–E, based on the material taken from different localities, appears having peaks at largely corresponding position between each other. Summing up all the data, the frequency distribution appears with 8 peaks as shown in the Fig. 5, F. Each of the peaks from the first to the eighth is standing at positions, 0.9–1.2, 2.4–2.7, 3.0–3.3, 3.9–4.2, 5.1–5.4, 5.7–6.0, 6.3–6.6 and 7.2–7.5 mm respectively.

(5) Relationship between body-length of the fish and number of resting zones of its scale

The number of the resting zones found on the scales runs as being 0–7 at least within the limit of the present material. Number of specimens examined and range average of the body-length assorted by localities from which the specimens were fished are given in the Table 3 in relation to the number of the resting zones. As seen from the Table 3, there is a gradual increase of body-length with increase of resting zone number in materials taken from Erimo in

Table 3. Frequency and body-length grouped by sampling time and locality in relation to resting zone number. *B.L.*, body-length in cm; *N*, number of individuals. Numerals laid within parenthesis give the average of *B.L.*.

Locality and date		Number of resting zones							
		0	1	2	3	4	5	6	7
Erimo	*N*		3	8	12	19	13	9	1
			19-24	17-24	16-26	16-24	14-25	18-25	19
Aug.	*B.L.*		(21.3)	(20.8)	(21.0)	(20.8)	(19.5)	(20.9)	(19)
Erimo	*N*			2	5	16	29	22	8
				24-25	21-26	20-25	19-27	19-27	20-25
Aug.	*B.L.*			(24.5)	(24.6)	(22.9)	(23.8)	(23.6)	(22.8)
Erimo	*N*		1	8	6	1			
			8	9-14	12-15	14			
Dec.	*B.L.*		(8)	(12.5)	(13.5)	(14)			
Chōshi	*N*	6	6	16	6	2			
		4-7	5-9	5-9	8-9	8-9			
Feb.	*B.L.*	(5.5)	(6.5)	(7.7)	(8.5)	(8.5)			
Hachinohe	*N*			1	2	3	5	4	5
				12	8-11	10-12	13-14	12-14	15-21
Feb.	*B.L.*			(12)	(9.5)	(10.6)	(13.4)	(13.2)	(17.2)

December, Hachinohe and Chōshi in February. But such trend is not found to exist for the materials obtained from Erimo in August. Frequency distribution of the actual body-length grouped by the number of the resting zones is shown in the Fig. 6 with very wide range of variation. The material, on which the Fig. 6 is based, is a material combined all specimens at my disposal including the specimens taken from the waters off Erimo in August. So that there is a wide range of variation of the body-length.

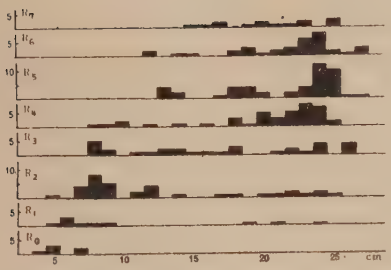

Fig. 6. Frequency distribution of body-length grouped by number of the resting zones. Abscissa, body-length in cm.

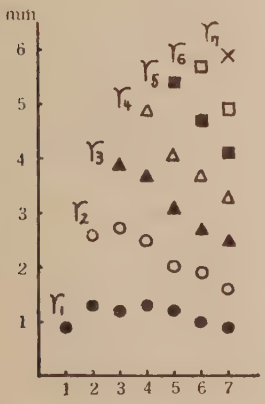

Fig. 8. Showing average distance of each resting zone of different age-groups. Ordinate, distance from the focus of scale in mm. Abscissa, age represented by resting zone number.

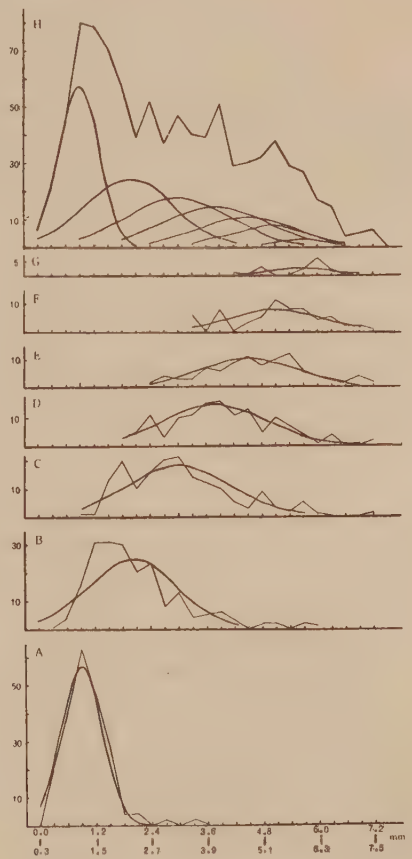

Fig. 7. Frequency distribution of r_1-r_7. Abscissa, distance from the focus of scale in mm. Smooth curves are normal curves fitted to the actual frequency data. A, r_1; B, r_2; C, r_3; D, r_4; E, r_5; F, r_6; G, r_7; H, total of r_1-r_7.

Frequency distributions of r_1-r_7 are given on the Fig. 7. The distributions show considerable variation except that of r_1, in which the distribution ranges within rather narrow limit as seen in the Fig. 7, A. But a progression of modes is present. Remarkably enough, each mode of r_1-r_7 is roughly in accordance with each peak of the frequency distribution of the whole resting zones of all the material dealt with (Fig. 7, H). The mean and standard deviation of r_1-r_7, and body-length corresponding to r_1-r_7 are shown in the Table 4. The mean of r_1-r_7 runs as follows; 1.1, 2.2, 3.2, 4.0, 4.7, 5.3, 5.9 cm respectively. Calculated body-lengths against each mean of r_1-r_7 are 5.23, 8.68, 11.49, 14.94, 17.02, 19.10 and 21.21 cm respectively.

Table 4. Mean ($\bar{r}$) and standard diviation (SD) of r mean ($\bar{L}$) and $\bar{L}\pm 2SD$ of the body-length corresponding to each r.

r	r (cm)	SD (mm)	$\bar{r}\pm 2SD$ (mm)	$\bar{L}$ (cm)	$\bar{L}\pm 2SD$ (cm)
r_1	1.1	0.43	0.24~1.96	5.23	2.9~ 7.9
r_2	2.2	1.01	0.18~4.22	8.68	2.7~15.3
r_3	3.2	1.15	0.90~5.50	11.49	4.7~19.8
r_4	4.0	1.14	1.72~6.28	14.94	7.1~22.5
r_5	4.7	1.10	2.50~6.90	17.02	9.6~24.7
r_6	5.3	0.99	3.32~7.28	19.10	12.3~26.1
r_7	5.9	0.68	4.54~7.26	21.21	16.4~26.0

It should be added herewith that an evident Lee phenomenon is presented regarding averages of r_1-r_7 as illustrated in the Fig. 8.

Size and age-relationship of this fish has been studied by the Hachinohe Branch of Tōhoku Sea Regional Fisheries Research Laboratory.[3] According to the result of the study made by the Laboratory, the body-length corresponding to each age, 1–6, is 7.4–11.2, 11.9–15.9, 18.1–19.3, 20.6–21.8, 23.5–25.1, 26.8–29.6 cm on range and 9.3, 13.7, 18.7, 21.2, 24.3, 28.2 cm on average respectively. But the method of age-determination employed by the Laboratory is not stated. The average of calculated body-length taken by the present investigation largely tallies with that of 1–, 2–, 3-age obtained by the Hachinohe Branch Laboratory in even-numbered r (r_2, r_4, and so on) only.

Conclusively the age-determination of this fish is no easy task at least by the following three reasons :—(1) The scale of this fish gives very complex picture, accordingly age-reading is hardly possible; (2) exact determinations of the time of yearly ring formation is impossible owing to the annuli are less defined; and (3) the spawning season of this fish covers a long range of period. All of these items enumerated above are, however, attributed to those being beyond human power. Therefore it is proposed to examine the age-determination of this fish in future from different angles of scrutiny with a view to work out this theme.

Summary

(1) Relationship between body-length (L) and scale-length (S) of this fish may be expressed by the following formula : $S=0.410(L-2.3)^{0.907}$.

(2) Resting zone formation of scale appears to take place twice in a year during such two seasons, one in months of May and June, the other in months of November and December.

(3) Eight peaks are found to exist in the frequency distribution of r of the whole material dealt with. The peaks at positions of 0.9–1.2, 2.4–2.7, 3.0–3.3, 3.9–4.2, 5.1–5.4, 5.7–6.0, 6.3–6.6, 7.2–7.5 mm from the focus of scale.

(4) The mean position of each resting zone from r_1 to r_7 is 1.1 ± 0.43, 2.2 ± 1.01, 3.2 ± 1.15, 4.0 ± 1.14, 4.7 ± 1.10, 5.3 ± 0.99, 5.9 ± 0.68 mm from the focus of scale.

(5) The body-length corresponding to the mean values of r_1-r_7 are 5.23, 8.68, 11.49, 14.94, 17.02, 19.10, 21.21 cm respectively.

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Journal of the Tokyo University of Fisheries, Vol. 40, No. 1, 1953

ON TWO NEW SPECIES OF PARASITIC COPEPODS OF MOLLUSKS

Toshikazu Hoshina and Yasuo Sugiura

(Received June 15, 1953)

Researches on parasitic copepods of the Mollusks of Japan were made by Mori (1935) and Yamaguti (1936), and, up to the present, only a small number of species have been recorded.

In the course of the study of the larval trematode, *Gymnophalloides tokiensis*, infesting the Japanese common oyster, the senior author had an opportunity to find a species of parasitic copepods in the oyster and another in a species of gastropoda, *Tegula* (*Omphalius*) *pfeifferi* (Phillippi). These two species, apparently new to science, form the basis of the descriptions which follow.

The authors have the pleasant duty of acknowledging their indebtedness to Professor Dr. Satyû Yamaguti of Okayama University, for his invaluable advice in the determination of the species and for supplying them, on loan, with necessary literature from his private library.

They are also grateful to Messrs. K. Konno, T. Masuda and J. Senô of Tokyo University of Fisheries, for their help in collecting the specimens of *Tegula* (*Omphalius*) *pfeifferi*.

1. *Parapanaietis tegulae* n. g. n. sp.

(Pl. 1. Figs. 1–6)

Thirteen females of the present species were found in the mouth cavity of *Tegula* (*Omphalius*) *pfeifferi* (Phillippi) in twelve specimens out of forty five of the *Tegula* species, collected on May 3, 1953 at Kominato, Chiba Pref..

Female: Body light orange or grayish, 2.6~3.4 mm long, broadest at first thoracic segment. Head fused with first thoracic segment, 0.28~0.38 mm long, 0.44~0.52 mm broad, with nauplius eye and a median ridge; frontal margin slightly convex; lateral margins divergent posteriorly. Nauplius eye 0.13~0.20 mm from frontal margin, at the level of mandible. Cephalothorax nearly trapezoidal, 0.47~0.59 mm long by 0.65~0.70 mm broad. Thorax 1.15~1.74 mm long; second to fifth thoracic segments free; second to fourth segments with almost same width, measuring 0.42~0.98 mm, but fourth segment somewhat larger than second, third and fifth segment; fifth segment smallest, measuring 0.14~0.39 mm long by 0.36~0.39 mm broad. Each segment arched dorsally and convex laterally.

Genital segment much smaller than fifth thoracic segment, 0.15~0.22 mm long by 0.18~0.31 mm broad, with convex sides.

Egg case 0.50~0.94 mm long, 0.19~0.20 mm broad, easily detachable, eggs small and rounded, 0.08~0.10 mm in diameter, arranged in a few longitudinal rows.

Abdomen 4-segmented, nearly cylindrical, tapering posteriorly, 0.65~0.68 mm long; first segment 0.23~0.26 mm broad; second 0.20~0.23 mm; third 0.15~0.19 mm; fourth 0.14~0.18 mm.

Caudal-rami uniramose, cylindrical, slightly tapering toward distal end, 0.14~0.15 mm long and 0.05~0.06 mm broad at the base, with a seta at middle of outer side and five apical setae one of which is long.

First antenna 7-segmented, gradually tapering distally, 0.20~0.23 mm long by 0.03~0.05 mm broad at base; first to sixth segments with 20~25 setae, most of which are on the antero-ventral margin; terminal segment small, with 5~6 setae at tip. Second antenna prehensile, 4-segmented; first segment enlarged with a setiform spine at distal end of inner margin; second stout, with well developed muscle in it and with one setiform spine at distal portion of inner margin; third short with two setae on inner margin; fourth also short, with a fine seta at distal end of outer margin, one seta on inner margin and two stout false-jointed terminal claws, one of which is large, about 0.06 mm long and the other small, about 0.04 mm long.

Mandibular blade finely serrated on inner and proximal outer margin, distal portion filamentous, with a small pectinate ramus.

First maxilla short and cylindrical with three setae on tip. Second maxilla stout at base; distal segment terminating in two pectinate rami.

Maxilliped cylindrical, 3-segmented; first to second segments almost same length; terminal segment small and conical with a digitiform process.

First to fourth pairs of legs biramose; basipod 2-segmented, each distal basipod with a seta at inner end; each endopod 2-segmented, exopod 3-segmented with exception of fourth leg, which consists of two or three segments.

The arrangement of spines and setae on each ramus of first to fourth legs is as follows:* First exopod 1~(0), 1~(0), 3~(3); endopod 0~(0~1), 3~(2); third exopod 1~(0), 0~1(0~1), 2~(2); endopod 0~(0~1), 2~(1); fourth exopod 1~(0), 0~(1), 3~(2); endopod 0~(0~1), 2(0).

Fifth leg uniramose, 1-segmented, cylindrical, with two short setae at its rounded tip and a seta in front of its base. Length 0.04~0.05 mm; breadth 0.02~0.03 mm at base.

Remarks: The present form is closely allied to the genus *Panaietis* STEBBING, 1900, from which it is distinguished by the differences as given in the following table (Table 1).

* Numerals put in parenthesis indicate the number of setae.

Table 1

Body parts	genus *Panaietis*	Present form
Head	Without nauplius eye	With nauplius eye
Genital segment	About same size as fifth thoracic segment	Much smaller than fifth thoracic segment
Abdomen	Comparatively well developed	Reduced
Second antenna	3-segmented, with 4 apical claws	4-segmented, with 2 apical claws
Mandibular blade	Proximal portion armed with numerous teeth	Without teeth
Second maxilla	With teeth	Without teeth
Maxilliped	Reduced	Well developed
First to fourth thoracic legs	Well developed; biramose, each ramus 3-segmented	Reduced; biramose exopod 3-segmented with exception of fourth exopod, which is 2 or 3-segmented; endopod 2-segmented

The above-mentioned differences may be sufficient to justify the erection of a new genus, for which the name *Parapanaietis* is proposed.

Parapanaietis n. g.

Generic Diagnosis: *Clausiidae* Sars 1918.

Head fused with first thoracic segment, with nauplius eye and a median ridge. Breadth of body largest at first thoracic segment. Second to fourth thoracic segment free. Genital segment much smaller than fourth thoracic segment, with convex sides. Egg case long; eggs numerous, arranged in a few rows.

Abdomen short, slightly tapering posteriorly. Caudal rami slightly narrowed posteriorly, tipped with five setae.

First antenna 7-segmented, gradually tapering distally, moderately armed with setae. Second antenna prehensile, 4-segmented, with two apical claws, each of which is false-jointed. Mandibular blade finely serrated on inner margin and proximal outer margin with a setiform spine on inner side of its base. First maxilla with three spiniform setae at tip. Second maxilla broad based; distal segment terminating in two pointed pectinate rami. Maxilliped moderatory developed, cylindrical, 3-segmented; terminal segment small, with a degitiform process. First to fourth legs biramose; basipod 2-segmented; each endopod 2-segmented; exopods 3-segmented with exception of fourth which consists of two or three segments. Fifth leg uniramose. Male unknown. Parasitic in marine gastropod.

Genotype. *Parapanaietis tegulae.*

2. *Myicola ostreae* n. sp.
(Pl. II. Figs. 7–14)

The present parasite was found on the surface of gills of *Ostrea gigas* Thunberg, inhabiting the sea shore of Yawata, Chiba Pref.. Twelve females of the parasite were collected as shown in following table (Table 2).

Table 2

Dates	Nos. of oyster examined*	Nos. of oyster infected	Nos. of the parasite collected
May, 4, 1951	20	2	2
Sept., 10, 〃	12	2	2
Nov., 13, 〃	20	6	8

* Shell size—Length, 5.0~6.6 cm; Altitude, 3.0~4.2 cm; Depth, 2.3~3.5 cm.

Female: Body plump, with greenish, wide digestive tract, 1.40~2.23 mm long by 0.45~0.62 mm broad at first thoracic segment. Head indistinctly separated from first thoracic segment, with nauplius eye, 0.25~0,29 mm long, 0.36~0.37 mm broad; frontal margin rounded; lateral margins widely divergent posteriorly. Nauplius eye blue, 0.06~0.09 mm from frontal margin.

Thorax large; second to fourth thoracic segments free with almost uniformly wide; first to fourth segments 1.00~1.58 mm long; fifth segment abruptly decreased in size, 0.13~0.20 mm long, 0.18~0.25 mm broad.

Genital segment 0.09~0.16 mm long by 0.15~0.21 mm broad, with convex sides. Egg case long elliptical, 0.76~0.85 mm long by 0.25~0.36 mm broad. Eggs large rounded, 0.12~0.19 mm 0.10~0.15 mm in diameter.

Abdomen small, 3-segmented, 0.23~0.27 mm long, 0.14~0.16 mm broad at the first segment and 0.08~0.09 mm broad at the terminal segment.

Caudal rami tapering distally, 0.09~0.11 mm long by 0.02 mm broad at base, with two setae on outer side and three setae at tip.

First antenna 7-segmented, 0.16~0.17 mm long by 0.03~0.04 mm broad at base; first segment with 3~7 setae; second 8~11; third 4~5; fourth 3~4; fifth 3~5; sixth 1~2, seventh 5~6, of which 3~4 setae are at the tip.

Second antenna prehensile, 3-segmented; basal segment stout; second segment short; third segment about two times as long as second segment, arcuate, with an apical claw about 0.08 mm long.

Mandible terminating in two slender setiform rami, finely serrated on inner margin, with a seta at proximal inner side.

First maxilla, short cylindrical, with two long setae and a short seta at tip.

Second maxilla, stout at base; distal segment terminating in two setiform pectinate rami, which are about the same in length. Maxilliped lacking.

First to fourth legs biramose; basipods 2-segmented with a seta at inner distal side. Each ramus 3-segmented, with minute spines on outer margin. The arrangement of setae on the first to fourth legs is as follows: First exopod 1, 1~2, 8~9; endopod 1, 1~2, 6; second exopod 1, 1, 8~9; endopod 1, 2, 6; third exopod 0~1, 2~3, 7~8, endopod 1, 2, 4~6; fourth exopod 1, 1~2, 8; endopod 1, 2, 4~5.

Fifth leg uniramose, 2-segmented, plate-like; basal segment with a seta at outer distal side, fringed with small spines on outer margin; terminal segment about twice as long as broad, with four setae on terminal margin. Total length 0.09~0.12 mm.

Remarks: The present species may be refered to the genus *Myicola*

Wright, 1885, from the above-described characters. Up to the present, two species have been recorded for this genus viz. *M. metisiensis* Wright, 1885 and *M. major* (Williams 1932).

The present new species is well distinguished from these species by the differences in thoracic and abdominal appendages.

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PLATE I

Figs. 1–6. *Parapanaietis tegulae* n. g. n. sp.

Fig. 1. Female, ventral view.

Fig. 2. First antenna of female, ventral view.

Fig. 3. Second antenna of female, ventral view.

Fig. 4. Mouth parts of female, ventral view. Md mandible; Mx^1 first maxilla; Mx^2, second maxilla; Mp maxilliped.

Fig. 5. I-V. First to fifth legs of female, ventral view. I first leg, II second leg III third leg, IV fourth leg, V fifth leg.

Fig. 6. Caudal rami of female, ventral view.

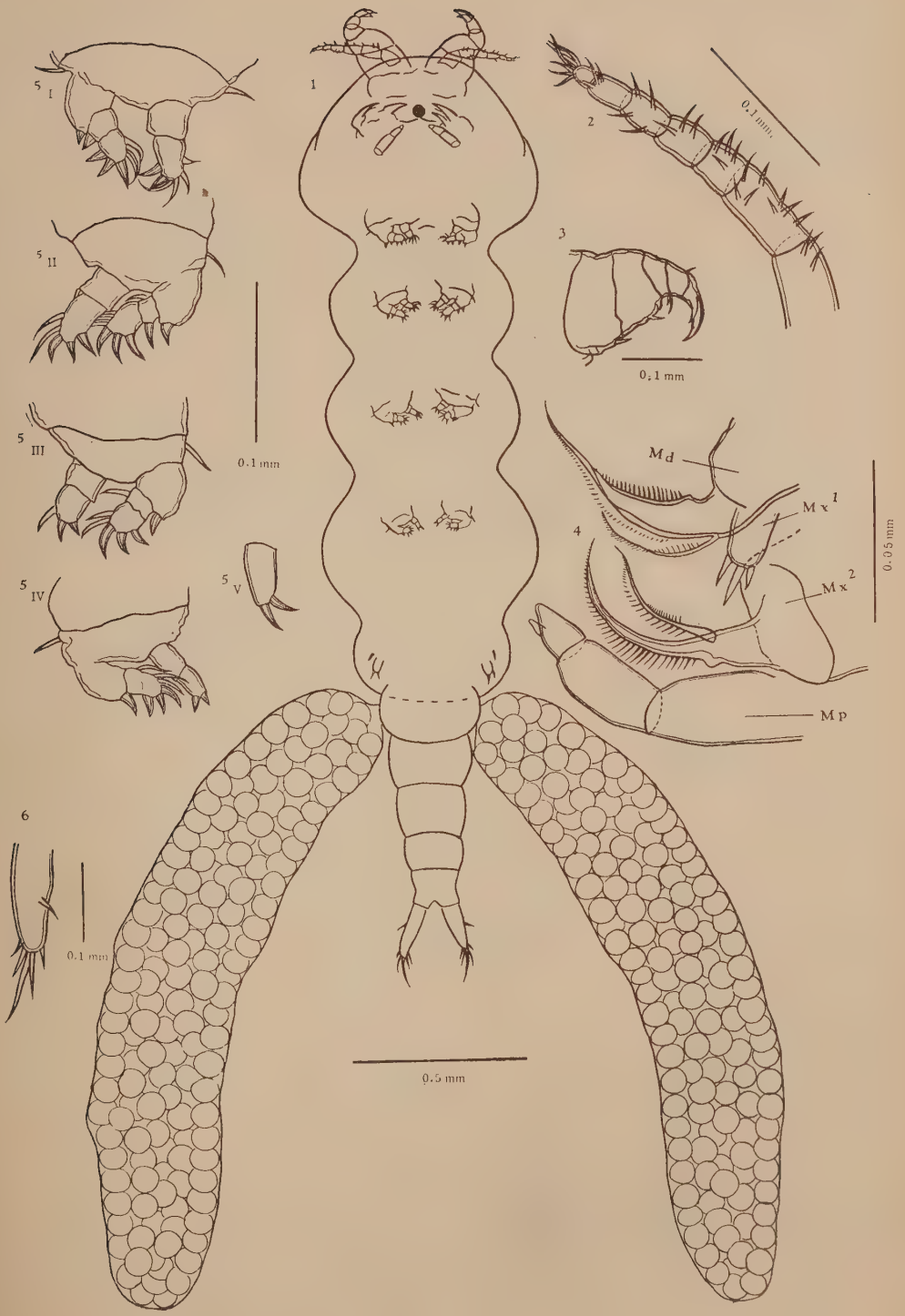

1
2
0.1 mm
3
0.1 mm
4
Md
Mx 1
Mx 2
Mp
0.05 mm
5 I
5 II
5 III
5 IV
5 V
0.1 mm
6
0.1 mm
0.5 mm

PLATE II

Figs. 7–14. *Myicola ostreae* n. sp.

Fig. 7. Female, dorsal view.

Fig. 8. First antenna of female, ventral view.

Fig. 9. Second antenna of female, ventral view.

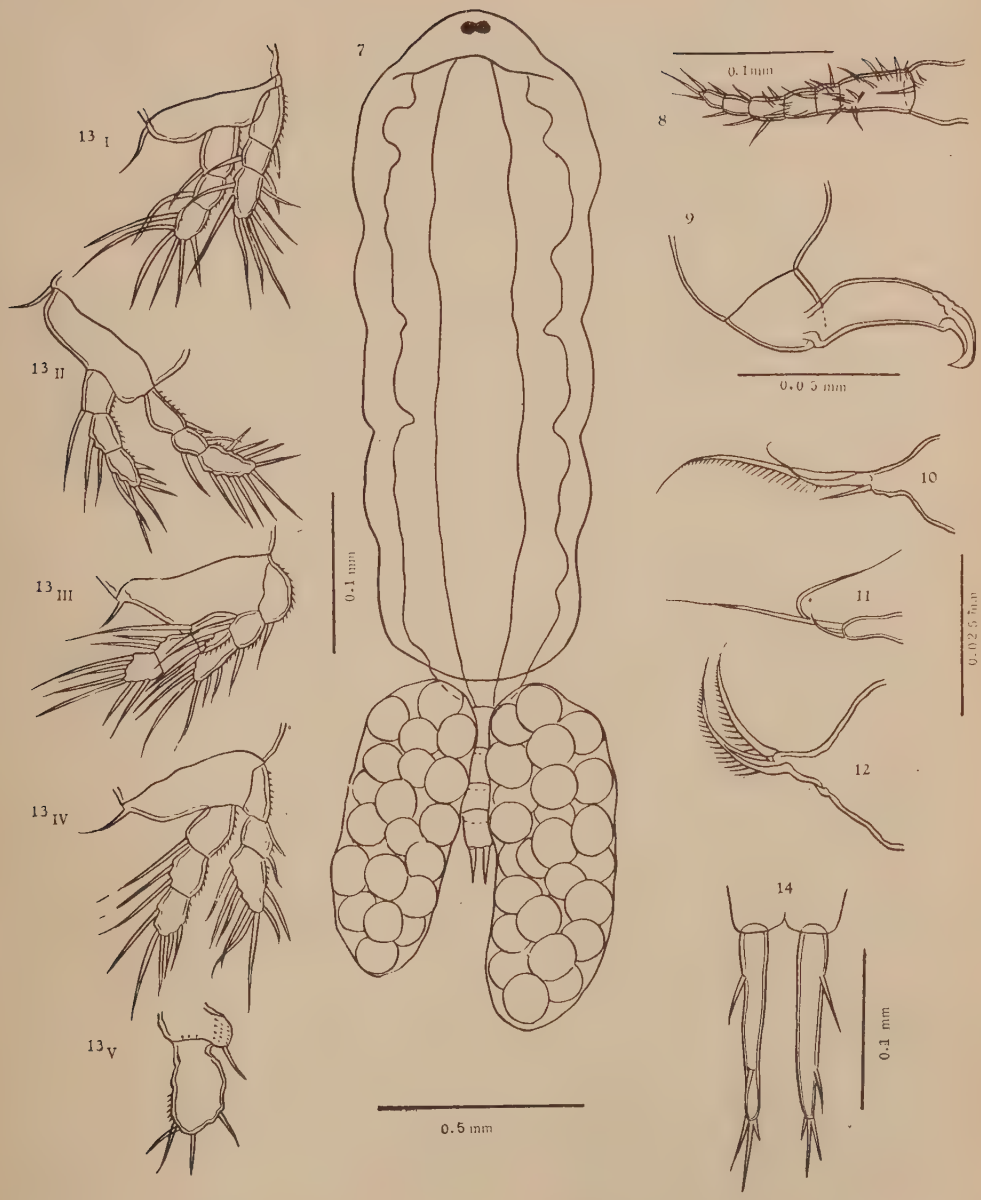
Fig. 10. Mandible of female, ventral view.

Fig. 11. First maxilla of female, ventral view.

Fig. 12. Second maxilla of female, ventral view.

Fig. 13, I-V. First to fifth legs of female, ventral view. I first leg, II second leg, III third leg, IV fourth leg, V fifth leg.

Fig. 14. Caudal rami.


13 I
13 II
13 III
13 IV
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7
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0.5 mm
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11
0.025 mm
12
14
0.1 mm

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HISTOLOGICAL AND CHEMICAL STUDIES ON THE FAT METABOLISM OF FISH
1. CHANGES IN THE MUSCLE FAT CONTENT DURING THE GROWTH OF THE CARP*

Toyoki Ono, Jirō Senō, Kenjirō Konno and Fumio Nagayama

(Received June 23, 1953)

The spawning is one of the important physiological phenomena in the life cycle of fish. At this stage the fish becomes lean as a result of the increased consumption of energy without food intake. Accordingly, a marked decrease in the content of the nutrients in muscle such as protein and fat, especially the fat component, is induced. The fundamental problem on the fat metabolism of fish may be raised: In what part of the body does such a change in fat content occur, and what constituents of the fatty substances are selectively utilized or synthesized, and by what process is the body fat formed and stored?

Biochemical studies on the body fat of fish have hitherto been made as to its constitution and content under the various physiological conditions. The works of Lovern,[1] Hilditch,[2] Mc Cay,[3] Rittenberg,[4] Tunison,[5] Schohenheimer,[6] and Ono[7] are concerned with fat constitution, and the reports on mackerels by Hasegawa,[8] Miyama and Saruya,[9] Miyama and Osakabe,[10] Uno and Nakamura,[11] and Ono,[12] and those on sardines by Oku,[13] Dill,[14] Yamashita,[15] Fukuoka[16] and Ono[17] are chiefly concerned with fat content. These results obtained by the above mentioned authors will be summarized as follows.

The constitution of the fatty acids in the fish fat is changed according to the diet. For example, the ordinary cultured eel or the eel fed with herring oil or other fish oil had always the higher content of the unsaturated fatty acids than did the natural eel. When the eel was cultured in the cool water the body weight was found to increase by 16 per cent, while the eel in the warm water showed an increase of 28 per cent. In the former case, the unsaturated fatty acid content was always higher than in the latter. The content of unsaturated fatty acids of the eel in herring oil was found to rise with the increase of the body fat. Further, it was also determined that the composition of fat was not always the same as the fatty acid composition of the diet, when the fishes were fed *Calanus finmarchicus*, a kind of plankton.

Concerning the seasonal variation of the fat content in the fish body, many experiments were carried out on mackerel and other Japanese marine fishes, in which it was found that the fat content differed considerably between the periods before and after spawning. In addition to this fact, Ono showed that the fat content was subjected to greater change in the ventral parts by the spawning than in other parts.

Sekine et al[18] made the biochemical research on salmon at stages from

* Aided by a grant from the Ministry of Education.

hatching to larva and observed that the body components of salmon was consumed during these stages, but that in the latter larval stage (heavier than 0.5 gram), the loading of fat increased rapidly in the body.

Greene[19] studied histologically on the distribution of muscular fat of king salmon, and found that the fat is stored in the body during the stage of feeding and that the growth became maximum at the beginning of the spawning. It was also shown that in the active caudal muscle the liposomes were decreased constantly and were often completely absent, as the advanced stages of exhaustion began to appear. The pink muscle fibres are plumb and cylindrical at the time when the migration begins having a high content of fat, but at the spawning time the large portion of fibres has the appearance of being shrunken due to the decrease of fat. He noted that the dark muscle is characterized by the enormous loading of intramuscular fat at the normal stage in contrast to the pink muscle.

It will be considered that these experiments represent some general pattern found in the fat metabolism in the fish body. In the present study an attempt was made to determine the site of fat storage during the stage of growth, and to observe the histological and chemical changes of the fat and the allied substances taking place at the stage of spawning, and at the same time to find out the relations between the distribution of calcium or phosphorus and the storage of fat.

In this paper the results of the histological observations and chemical data on the fat distribution have been given for carps.

Our thanks are due to Mr. Jusaburō Konno, the director of the Tama Ryūsui Yōgyojō (Tama Running Water Fish-farm), for his kind supply of living carps for this work.

We must express our hearty thanks to Dr. Takashi Fujii, Prof. of Tōkyō University, for his kindness in reading the original manuscript.

EXPERIMENTAL

1. Material

Carp was used as a sample of the fresh water fish and mackerel as a representative marine fish. The carps at various stages from larva to young fish were used and the sample was each divided into three or four parts as illustrated in figure 1 in the previous report.[12] After the removal of scales from each part, the determinations of the moisture, fat and phosphorus were made. Other parts were fixed in 10% formalin solution for histological observation.

As it was difficult to obtain the mackerel of disired sizes as in the case of carp, the samples of small, middle and large sizes were selected from a fish market.

2. Histological observation of the body fat

The formalin fixed materials were washed in running water for a proper time according to size and a slice was prepared by the freezing method using

dry ice. Even the comparatively large samples for section are frozen within 20～30 minutes with dry ice. After dipping each slice in 50 per cent alcoholic solution for several minutes, it was placed in 70% alcoholic solution of Sudan III for about a half minute. Then it was transferred into 50% alcoholic solution, and in distilled water for a few minutes. Finally it was passed through a filtered dilute solution of Mayer's haemalum, washed in alkaline tap water for several minutes and was mounted in glycerol jelly.[20]

3. Chemical determination

The fish body was divided into three or four blocks, and each block obtained from 3～5 individuals at various stages was respectively ground after the removal of scales, and was analysed.

Moisture and fat were determined by the standard method and also analysis was made for water-soluble, water-insoluble and fat-soluble phosphorus. The water-soluble phosphorus was determined by the Tisdall's coloric method with a protein free extract which was obtained from the fat-free samples. The water-insoluble and fat-soluble phosphorus were estimated using the nitric acid solution obtained from the ash of sample. The results on the water-soluble phosphorus were agreed with those obtained by the standard method.

RESULTS

I. Histological observations

A. Dorsal portion

As shown in Figure 1 (Plate III), the deposition of fat in carp's young seems to occur mainly in the connective tissue around the vertebrae. The fat droplets were also found in the connective tissue near the active region such as anterior dorsal supracarinal muscle. The adipose tissue was hardly observed in the dorsal muscle in this stage.

The dark muscle localized right under the lateral line which is rich in blood, was also found to contain much fat already in young fishes, in sharp contrast to other muscle tissues, which contain little fat in this stage (Pl. III, Fig. 3).

The content of fat in the immature fish increased rapidly not only in quantity but also in histological appearance, namely as shown in Fig. 2 (Pl. III). The subcutaneous tissue with fat droplets enlarged greatly as compared with that of young carp (Pl. III, Fig. 4). However, the increase in the amount of fat deposit was not marked in other ordinary muscle tissue.

B. Ventral portion

Our previous work[12] has shown that the ventral muscle of fish shows abundant fat deposition in general and that its amount fluctuates according to the stages, especially before and after spawning. However, the loading of fat in the abdominal portion was found rather poor in the case of young carp, fat

being observed merely in the connective tissue which surrounded the anterior ventral infracarinal muscle (Pl. IV, Fig. 5).

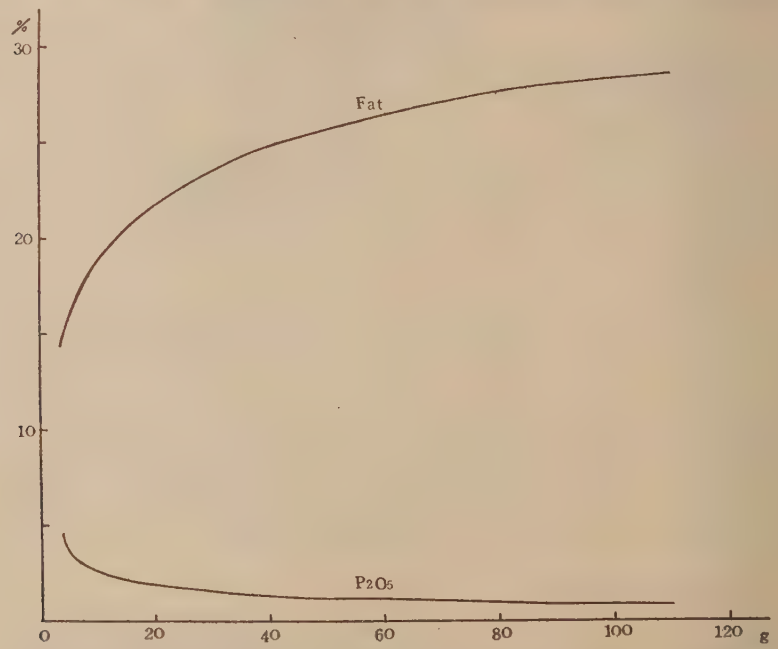
On the contrary, the storage of fat increased rapidly in immature fish and the enormous fat was stored in the well developed subcutaneous adipose tissue as shown in Plate IV-Fig. 6.

C. Caudal portion

It is generally known that the active tissue such as caudal muscle contains relatively small amounts of fat because of the active motion at the time of migration. In carps this moving function is also naturally observed, but in young individuals the content of body fat was very low as compared with the immature fishes. Therefore, the differences due to functional activity did not obviously appear in the case of carp either in young or immature.

The deposition of fat around the vertebrae in caudal portion was not so remarkable as was found in the dorsal region. The fat content of dark muscle was also low owing to the poor development of the dark muscle in the caudal.

It may be summarized from the results of the histological observations that the main sites of the distribution of body fat in carp's young is the connective tissues near the basal region of the dorsal fin, the same tissue near the anterior ventral infracarinal muscle, and the surrounding tissue of the vertebrae.



Text-fig. 1. Average contents of fat and phosphorus in carp muscles during the growth. Ordinate, contents (%) in dry matter; Abscissa, body weight in grams.

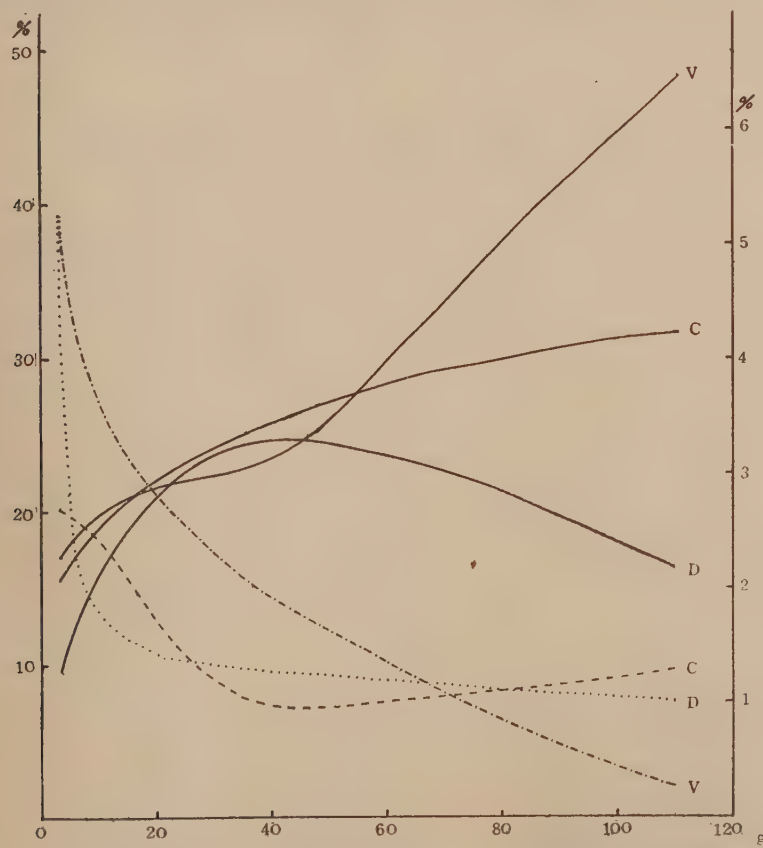
The fat content of muscular tissue is on the whole low except in the case of the dark muscle.

In the case of immature fish, however, the fat storage increased rapidly in the ventral muscle, the increase in the caudal muscle being least as compared with that of the dorsal and ventral muscle.

II. Chemical observations

A. The fat content

The fat content of carp muscles increased during the growth as shown in text-figure 1. In the case of carp's young, the loading of fat in the ventral portion indicates somewhat higher values than those of dorsal and caudal regions



Text-fig. 2. Fat and phosphorus contents in the divided parts of carp muscles. Left ordinate, fat contents (solid line, %) in dry matter; right ordinate, phosphorus content ("dots-dashes," broken and dotted lines, %) in dry matter; Abscissa, body weight in grams; V, ventral portion; C, caudal portion; D, dorsal portion.

(Tab. 1 & Text-fig. 2). When the fish reaches immature stage, the fat content increases rapidly in every portion, especially in abdomen, storing larger amount of fat (Tab. 1 & Text-fig. 2). These results agree with those of histological observations.

The comparison of fat content at the young and immature stages do not show marked differences between caudal and other portions, as was the case with mackerel.[12] (Tab. 1). Therefore, it seems that the caudal portion of young carp may occasionally show higher fat content than other portions (Tab. 1).

B. The phosphorus content

The phosphorus in fish muscle is contained as inorganic or organic compounds and its physiological function concerning the various metabolism has been studied by many authors. We have attempted to study what role has the phosphorus in fish muscle during the fat metabolism.

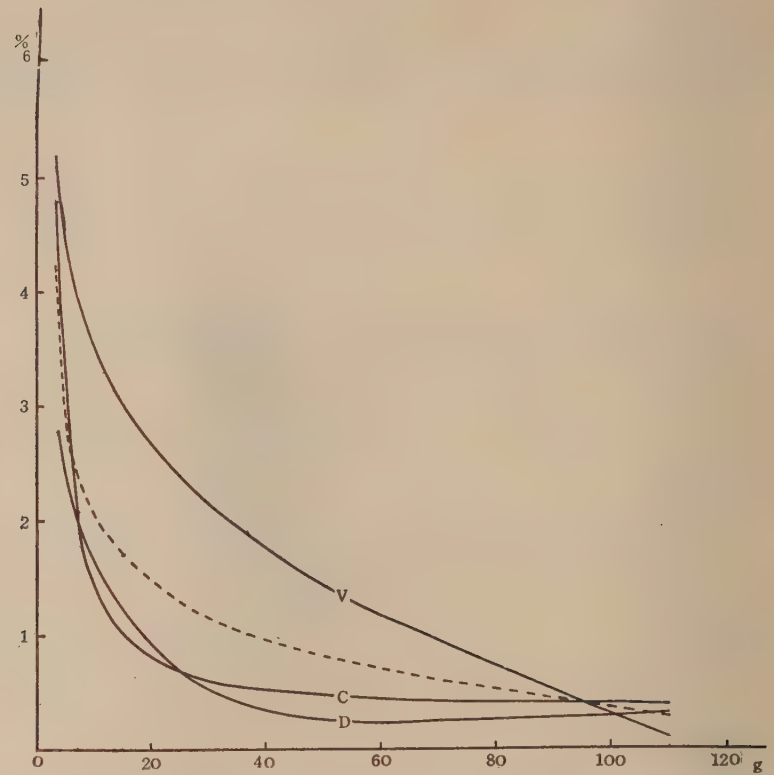
Table 1. Fat and phosphorus contents in the divided carp muscles. (D: Dorsal portion, D-A: Antero-dorsal portion, D-P: Postero-dorsal portion, V: Ventral portion, C: Caudal portion)

Sample number	Numbers of individuals	Average body length (mm)	Average body weight (g)	Body division	Moisture (%)	Fat (%)		Phosphorus (%)	
						Fresh matter	Dry matter	Fresh matter	Dry matter
1	5	50	3.6	D	77.31	2.17	9.55	0.85	2.74
				V	76.00	4.22	17.73	1.22	5.10
				C	77.03	3.66	15.78	1.21	5.26
	Average				**76.78**	**3.35**	**14.36**	**1.02**	**4.36**
2	3	62	7.7	D	76.10	3.43	14.71	0.62	2.60
				V	74.32	5.13	19.88	0.98	3.83
				C	72.75	5.49	19.80	0.44	1.62
	Average				**74.39**	**4.68**	**18.13**	**0.64**	**2.68**
3	3	77	15.0	D	72.58	4.31	17.82	0.63	2.31
				V	70.20	6.52	21.81	0.93	3.11
				C	74.16	5.51	20.32	0.38	1.48
	Average				**72.31**	**5.45**	**19.98**	**0.65**	**2.30**
4	3	127	36.8	D	77.52	5.56	24.70	0.23	1.02
				V	74.68	5.97	23.60	0.52	2.05
				C	77.23	5.87	25.60	0.32	1.39
	Average				**76.48**	**5.80**	**24.63**	**0.35**	**1.49**
5	3	158	110.0	D-A	75.60	4.53	18.50	0.31	1.29
				D-P	76.12	3.50	14.71	0.23	0.95
				V	63.78	17.80	48.60	0.10	0·28
				C	67.80	10.40	32.30	0.34	1.05
	Average				**70.82**	**9.06**	**28.53**	**0.25**	**0.89**

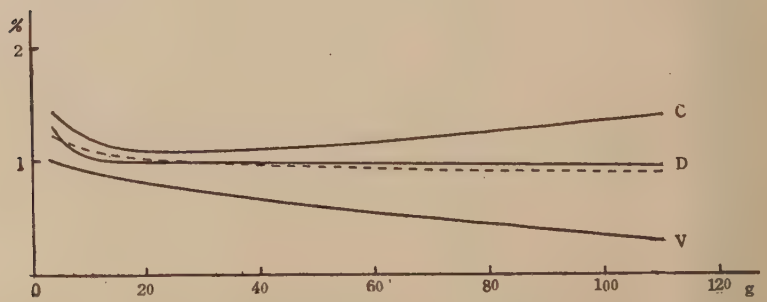
As shown in Table 2 and text-figure 1, the total phosphorus in fish body decreases in content with the growth, in contrast to a rise of fat content. The decrease in phosphorus content is greatest in the water insoluble-phosphorus (Text-fig. 3), and less in the water soluble- and fat soluble-phosphorus (Text-fig. 4).

Table 2. The phosphorus content of various types in the carp muscle. (I: In fresh matter, II: In dry matter, III: In fat free material, IV: In ether extracts)

Sample number	Body division	Water soluble phosphorus			Water insoluble phosphorus			Fat soluble phosphorus		
		I	II	III	I	II	III	I	II	IV
		%	%	%	%	%	%	%	%	%
1	D	0.2600	1.15	1.27	0.5750	2.53	2.79	0.0152	0.07	0.70
	V	0.2000	0.83	1.01	1.0150	4.26	5.17	0.0020	0.01	0.05
	C	0.2800	1.21	1.44	0.9050	3.92	4.67	0.0297	0.13	0.81
Average		0.2470	1.06	1.24	0.8320	3.57	4.21	0.0102	0.07	0.52
2	D	0.2600	1.04	1.11	0.3487	1.50	1.76	0.0367	0.16	1.07
	V	0.2600	1.04	0.72	—	—	—	0.0351	0.14	0.76
	C	0.2405	0.89	1.01	0.4373	1.60	2.07	0.0250	0.17	0.47
Average		0.2535	0.99	0.95	0.3930	1.55	1.92	0.0323	0.14	0.77
3	D	0.2600	0.97	1.05	0.3630	1.32	1.44	0.0055	0.02	0.25
	V	0.2400	0.81	1.07	0.6730	2.25	2.96	0.0141	0.05	0.20
	C	0.2350	0.91	1.10	0.1410	0.54	0.63	0.0065	0.03	0.18
Average		0.2450	0.90	1.07	0.3920	1.37	1.68	0.0087	0.03	0.21
4	D	0.1700	0.75	1.00	0.0530	0.23	0.31	0.0087	0.04	0.16
	V	0.1440	0.57	0.74	0.3690	1.46	1.91	0.0050	0.02	0.08
	C	0.2490	1.10	1.48	0.1420	0.62	0.84	0.0152	0.07	0.26
Average		0.1880	0.81	1.07	0.1880	0.77	1.02	0.0096	0.04	0.17
5	D-A	0.2180	0.89	1.09	0.0840	0.34	0.42	0.0134	0.06	0.30
	D-P	0.1700	0.73	0.85	0.0430	0.18	0.21	0.0098	0.04	0.28
	V	0.0630	0.18	0.34	0.0190	0.05	0.10	0.0230	0.06	0.12
	C	0.3030	0.71	1.38	0.0870	0.27	0.39	0.0210	0.07	0.20
Average		0.1885	0.63	0.92	0.0582	0.21	0.28	0.0168	0.06	0.23



Text-fig. 3. Changes in the contents of water-insoluble phosphorus during the growth. Ordinate, contents (%) in dry matter; abscissa, body weight in grams; solid lines, values for each portion of the divided body (V, C & D); broken line, average value of 3 (V, C & D) or 4 (V, C, D-A & D-P) divided portions.



Text-fig. 4. Changes in the contents of water-soluble phosphorus during the growth. Notices on ordinate, abscissa, solid lines, broken line and letterings are all the same as those in text-fig. 3.

SUMMARY

1. The fat loading of muscle in carp's young was merely observed in the connective tissue near the dorsal fin and around vertebrae, no special adipose tissue being formed in the muscle except in the dark muscle.
2. Owing to the development of adipose tissue in abdomen at an immature stage, the content of fat in this region rise more rapidly than any other portions.
3. As no tendency to an enormous decrease in the fat storage in caudal portion is observed in carp's young comparing with dorsal and ventral portions such as observed in the case of mackerel, the obtained high values for fat in the caudal portion may well be accidental.
4. Contrary to the increase of fat content, the total phosphorus in carp muscle declined corresponding to the growth, showing greater decrease in the water insoluble type and less diminution in the water soluble and fat soluble ones.

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PLATE III

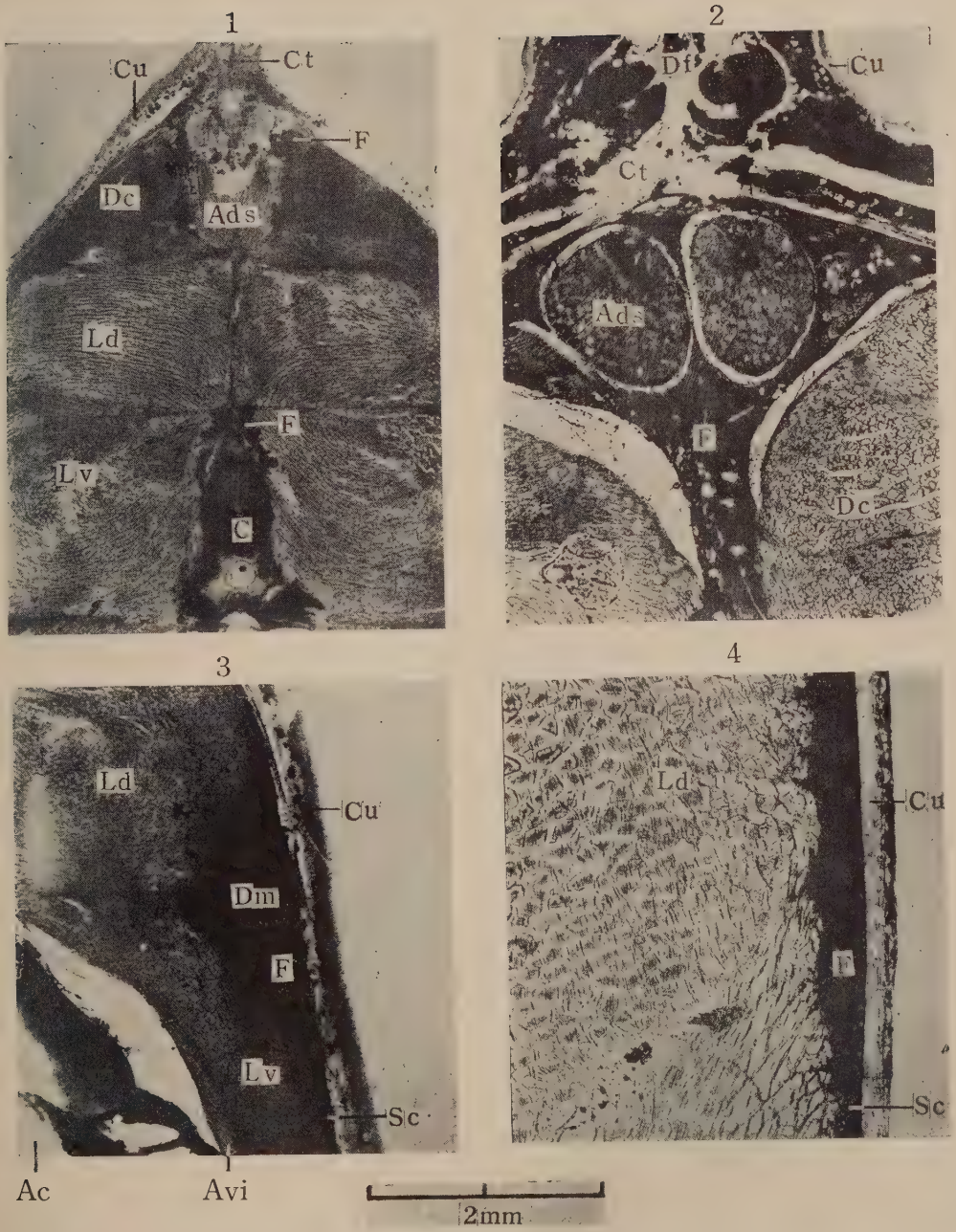
(Letterings in figures without notices are identical with those in foregoing ones)

Fig. 1. Showing the transverse section at antero-dorsal portion of a young carp, (sample A: 41 mm in body length, 2.5 g in body weight); Ct: connective tissue, Cu: cutaneous tissue, Ads: anterior dorsal supracarinal muscle, Dc: Dorsal carinate muscle, F: fat droplets, Ld: Latero-dorsal muscle, Lv: Latero-ventral muscle, C: Centrum, ×20.

Fig. 2. Transverse section at nearly the same portion as in Fig. 1 of an immature carp, (sample B, 158 mm in body length, 110 g in body weight); Df: dorsal fin. ×20.

Fig. 3. Transverse section of sample A at a layer a short distance posterior than that of Fig. 1. Dm: dark muscle, Sc: Subcutaneous tissue (=adipose tissue), Avi: Anterior ventral infracarinal muscle, Ac: abdominal cavity, ×20.

Fig. 4. Tranverse section of sample B at postero-dorsal portion, showing the enormous development of subcutaneous adipose tissue (Sc), ×50.


1
Cu
Ct
F
Dc
Ads
Ld
F
Lv
C
2
Df
Cu
Ct
Ads
F
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3
Ld
Cu
Dm
F
Lv
Sc
4
Ld
Cu
F
Sc
Ac
Avi
2mm

PLATE IV

(Letterings in figures without notices are identical with those in foregoing ones)

Fig. 5. Transverse section at postero-ventral portion of another young carp, showing characteristic storage of fat in the connective tissue at ventral margin; I: Intestine, Vc: Ventral carinate muscle, Mvi: Medial ventral infracarinal muscle. ×20.

Fig. 6. A part of transverse section at nearly the same portion as in Fig. 5 of sample B, showing the increase of fat storage due to the thickening of abdomen. ×20.

Fig. 7. Narrower optic field of the same preparation as in Fig. 6, highly magnified, showing a great number of adipose cells which contain fat to some extent at least with the various grades in staining tone. ×50.

Fig. 8. Transverse section at ventro-caudal portion of young carp, showing scanty adipose tissue therein. Vic: Ventral internal caudal pinna flexor muscle. ×20.

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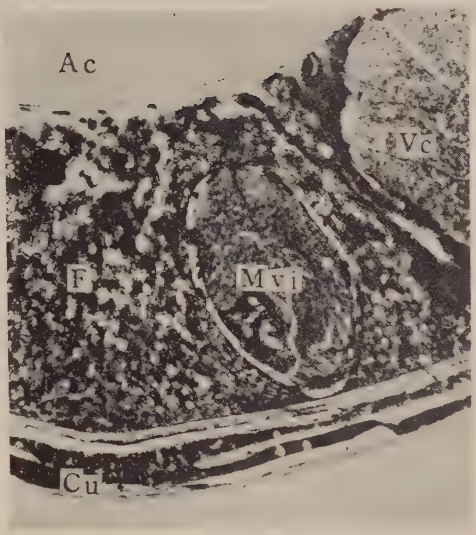

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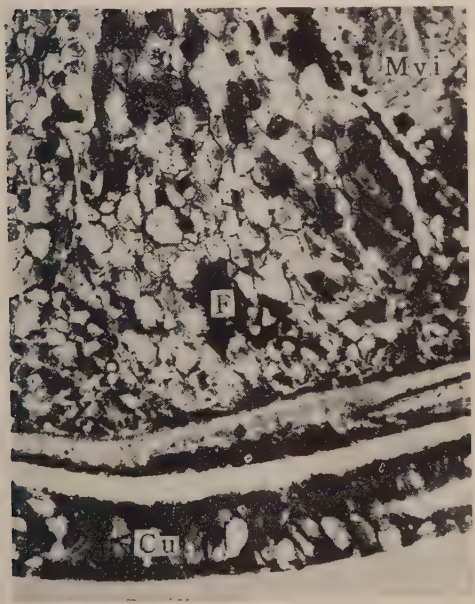

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STUDIES ON KAMABOKO-FORMING ABILITY OF MYOSIN

(Part III)

Hiroshi Hirano

(Received June 15, 1953)

V. THE TENABILITY OF DRY EUMYOSIN

V, 1. Introduction

As it has early been reported by the author, so-called powder of raw fish prepared by means of a centrifugal atomizer if left exposed to moisture falls off promptly in the content of a protein fraction such as is not only soluble in NaCl solution but coagulated by heat (Hirano, 1942 a). On the other hand, his discovery that thermal coagulation of eumyosin is virtually of cyclic reversibility, *i.e.*, that *Kamaboko*-formable protein can be recovered more or less completely even from its heat coagulum if the latter is once dissolved in an ice-cold solution of NaOH and the dissolved protein is reprecipitated by neutralization, implies that the progressive failure in the recoverability during dry-storage would be also but a physical phenomenon as will be illustrated below.

In the present chapter will be described in the first place the method affording the recovery estimation of eumyosin latent in its coagulum, next the rate at which the protein retires in resumability during dry-storage, and finally some discussions on the nature of the process through which dry eumyosin becomes spontaneously less-recoverable.

V, 2. Recovery Estimation of Eumyosin from its Coagulum

The method to estimate eumyosin recoverable from its coagulum wet or dry agrees in principle with that to isolate the protein from a routine preparation of bleached fish. It is based upon "protonogelatability" of eumyosin (*cf.* Hirano, 1953, p. 148), which has been proved to be the sufficient factor for *Kamaboko*-forming ability. Since the method consists of several stages of process, such as, extraction with alkali, precipitation at the isoionic point, protonogelation at a faintly basic reaction, *etc.*, these stages will be conditioned below one by one tracing them back from the last towards the top.

V, 2, 1. The Sufficient Condition for the Quantitative Flocculation of Eumyosin at pH 8.5

Table 27 shows the apparant solubilities of eumyosin which were found when subdivisions of an NaOH solution having equally contained 28.7 *mg* eumyosin N and 8.45 *mg* not-gelatable N per 100 *cc* were brought beforehand to pH 5.5 and kept at 35° C for two hours and brought subsequently back to pH 8.5 and kept again at 35° C but for various durations.

As seen from the table, the peptization and hence the protonogelation of

Table 27. The Effect of the Time of Maintenance at pH 8.5 upon the Recovery of Eumyosin from its NaOH-Solution.

Number of sample	No. 1	No. 2	No. 3
Time when the reaction was adjusted to pH 5.5	$8^h\ 50^m$	$8^h\ 55^m$	$9^h\ 0^m$
Time when the reaction was readjusted to pH 8.5	$10^h\ 50^m$	$10^h\ 55^m$	$11^h\ 0^m$
Time interval for which pH 5.5 was maintained	2.0 *hrs*	2.0 *hrs*	2.0 *hrs*
Appearance of sample {at pH 5.5 / at pH 8.5	Flocculums floated; mother liquors were hazy. Slightly turbid mother liquors with semi-transparent bulky precipitates.		
Time when centrifugation was performed*.....	$11^h\ 50^m$~$12^h\ 5^m$	$12^h\ 55^m$~$13^h\ 10^m$	$15^h\ 0^m$~15^m
Time interval for which pH 8.5 was maintained	1.0 *hr*	2.0 *hrs*	4.0 *hrs*
Appearance of centrifuged mother liquors {before filtration / after filtration	Hazy Trace of haze	Hazy Trace of haze	Hazy Trace of haze
Total nitrogen in filtrate (*mg*/25 *cc*)	2.11	2.08	2.10

* Vaporization loss of water during centrifugation proved to remain within the range of sampling fluctuation, *i.e.*, 0.6~0.7%.

eumyosin at pH 8.5, which has undergone such a preliminary treatment as above-mentioned, may be regarded to attain to an equilibrium within an hour at 35° C. In addition, the result that the amount of filterably dissolved N, being 2.10 *mg* per 25 *cc* or 8.40 *mg* per 100 *cc* on the average, stands quite in agreement with the initial amount of not-gelatable N points to that eumyosin may be precipitated to the utmost at pH 8.5 when kept at 35° C for an hour.

V, 2, 2. The Effect of Previous Maintenance of Eumyosin at pH 5.5 upon its Recoverability

Since it is presumable that, as already mentioned, eumyosin forms secondary fibrils if more readily at pH 5.5 than at pH 8.5 (*cf.* HIRANO, 1953, p. 166), the portion recoverable from its NaOH solution is expected to vary also with the duration for which it is kept precipitated at pH 5.5. There are given in Tables 28a and 28b relations between the recovery rate and the time of maintenance, which were found respectively when an eumyosin-NaOH solution was faintly acidified with HCl and kept at 7° C on the one hand and at 35° C on the other.

As it is evident from the tables, eumyosin recovered from its solution containing NaOH varies in amount depending upon the temperature as well as upon the duration of maintenance at pH 5.5. Thus, whereas the recovery rate does not yet attain to a maximum within 24 hours at 7°C, it does already in 15 minutes when the acidified system is kept at 35° C. Moreover, if the amount of the protein having undergone protonogelation is plotted against the time of maintenance at 7° C referring to Table 28a, a relation is obtained as shown by the

Table 28. Recovery of Eumyosin after Precipitation at pH 5.5 for Various Periods.

Number of sample	Time intervals during which samples were kept at pH 5.5 (*hr*)	Appearance of samples after they were kept at pH 8.5 and 35°C for an hour	Velocity of filtration	Turbidity of filtrate	Nitrogen (*mg*)†		Eumyosin recovery (%)§
					in filtrate (per 25 *cc*)	precipitated	
a: Samples were kept in an ice-box at 7° C.							
*a*1	0	Hazy liquor.	Very large	Hazy	9.02	0.05	0.7
*a*2	1	Slightly turbid liquor.	Ditto	Slightly turbid	8.98	0.09	1.3
*a*3	2	Ditto	Large	Ditto	8.86	0.21	3.0
*a*4	4	Ditto	Moderate	Ditto	8.70	0.37	5.3
*a*5	8	Contained a little flocculum.*	Small	Clear	4.32	4.75	68.0
*a*6	16	Contained a fairly large amount of flocculum.*	Very small	Ditto	2.68	6.39	91.3
*a*7	24	Ditto*	Ditto	Ditto	2.23	6.84	97.8
b: Samples were kept in an incubator at 35° C.							
*b*1	0	Slightly turbid, apparently uniform liquid.	Moderate	Faintly turbid	8.92	0.24	3.4
*b*2	0.25	Contained a large amount of flocculum; separated clear supernatant afterwards.	Extremely small	Clear	2.16	7.00	99.0
*b*3	0.50	Ditto	Ditto	Ditto	2.29	6.87	97.1
*b*4	1.0	Ditto	Ditto	Ditto	2.16	7.00	99.0
*b*5	2.0	Ditto	Ditto	Ditto	1.98	7.18	101.5
*b*6	6.0	Ditto	Small	Ditto	2.00	7.16	101.2

† Employing the values found for controls, it was assumed that the samples of each set were equal to one another in regard to total nitrogen content of which 2.08~2.09 *mg* were not-gelatable N.

§ The difference between the amount of total N and initial amount of the N incapable of gelation at pH 8.5 was regarded as 100%.

* Mother liquors in these samples were semitransparently hazy, appearing like wet matted-glass.

curve in Figure 21, which exhibits a typical course of consecutive reaction. This implies that isoionic eumyosin is first associated into small particles, presumably secondary fibrils, and these particles agglutinate afterwards further into large units undergoing protonogelation.

Now, it is concluded from the results of the preceding experiments, V, 2, 1 and V, 2, 2, that the following procedure will provide a successful method for qnantitive estimation of the eumyosin contained in a solution of NaOH: The solution is first acidified by adding HCl or acetic acid to pH 5.5 and kept at 35° C for an hour, after which the amount of the protein remaining precipitated is estimated as usual.

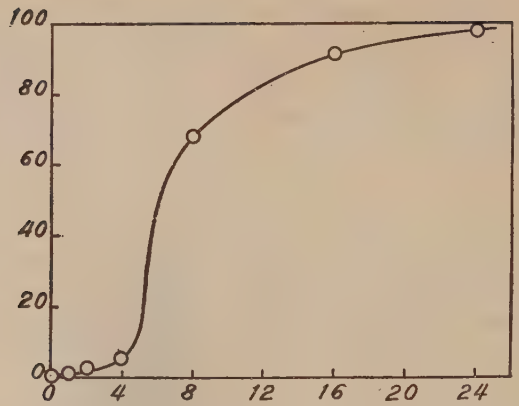

Fig. 21. The extent to which an isoionic precipitate of eumyosin is endowed with protonogelatability. Abscissa: Time of maintenance of the precipitate at 7° C (in *hr*). Ordinate: Protonogelatability endowed, as given in *per cent*.

V, 2, 3. The Effect of the Maintenance in NaOH-Solution upon the Recovery of Eumyosin

Eumyosin too is often decomposed when treated with a strong alkali, especially at a not-low temperature, liberating a part of its constituent nitrogen as ammonia. Even when such a drastic chemical change is not detected, the viscosity of an eumyosin sol containing NaOH decreases usually with the advance of time; and the sol yields no longer protonogelatable protein after its viscosity has once dropped beneath a certain extremity. From these facts, it may be expected that, when eumyosin is extracted with NaOH solution from its coagulum, its recovery amount will be influenced substantially by the pH, the temperature, and the period of extraction. With the retraction in protonogelatability, the protein becomes gradually more soluble in water at pH 8.5 where intact eumyosin behaves virtually as an insoluble matter in the practical absence of neutral salt. For example, the solubility estimated at 0.08 *mg* N/100 *cc* after a day rose up to 1.5 *mg* N/100 *cc* in 24 days during the storage at 2° C.

Table 29 furnishes another instance of such solubility change detected at pH 8.5; here the protein had been stored at pH 11.2 in an NaOH solution whose temperature was held at 12° C.

Table 29. Failure in Protonogelatability of Eumyosin Dissolved in NaOH-Solution and Stored at pH 11.2 and 12° C.

Number of sample	Period of storage (*hr*)	Nitrogen having remained dissolved at pH 8.5	
		(*mg*/100 *cc*)	(in per cent of total N)
1	1	1.41	28.5
2	3	1.69	34.2
3	24	3.11	62.8
4	48	3.91	78.8

Table 30. Recovery of Eumyosin from its Freshly Prepared NaOH-Solution at pH 11.8 Kept in a Refrigerator at 2° C.

Number of sample	Period of storage (*hr*)	Amount of nitrogen at pH 8.5 (*mg*)		Eumyosin recovery (%)	Remarks
		found in 100 *cc* mother liquor	calculated for precipitate		
1	0	0.05	32.10	100	Not treated with NaOH.
2	4	0.48	31.67	98.7	Recovered from NaOH sol.
3	8	0.60	31.55	98.2	〃
4	16	0.52	31.63	98.5	〃
5	24	0.64	31.51	98.1	〃
6	48	0.83	31.32	97.5	〃
7	72	1.25	30.90	96.2	〃

As seen from the table, the alkali-damage detectable on the recession in the precipitability at pH 8.5 develops fairly promptly even at a temperature as low as 12° C; so it is evident that the preparation of Na-eumyosinate solution should be carried out at a far lower temperature than 12° C if the damage is hoped to be evaded as completely as possible.

Table 30 exemplifes the recovery rate of eumyosin from its NaOH-solution at pH 11.8, which was kept in a refrigerator at 2° C.

Judged from this experimental result, eumyosin is to be recovered practically up to 100% even from its strongly basic solution having a pH as high as about 12 at least in one or two days immediately after the addition of NaOH in so far as the solution is kept at as low a temperature as 2° C.

It also should be remembered that an NaOH solution furnishing the extraction system with a basicity of about pH 12 is of sufficient strength if not of necessary for the exhaustive extraction of eumyosin from its deposit; and from such a point of view, it may be said that eumyosin has become unrecoverable if it behaves incapably of being extracted with such an NaOH solution as conditioned just above.

V, 2, 4. The Procedure for the Recovery Estimation of Eumyosin

Being based on the preliminary experiments from V, 2, 1 to V, 2, 3, the author now proposes a procedure as follows to estimate eumyosin recoverable from its coagulum:

The sample from which eumyosin is to be recovered be firstly soaked in a dilute solution of NaOH which has previously been cooled down to a temperature below 2° C. The amount of the NaOH solution employed be 4000 to 5000 times that of the total nitrogen contained in the sample (M *mg*). Adjust the extraction system to about 12 by pH, and continue extraction for 16 to 24 hours at a low temperature below 2° C. Total volume of the system, v_1 *cc*, should be recorded beforehand. Then, let the extract pass through a sheet of dry filter paper avoiding the agitation of insoluble residue if such is extant. Take up an aliquot portion of the filtrate (v_2 *cc*) in duplicate so that its total nitrogen content

(m *mg*) may be estimated on the one hand and such treatment as follows may be carried out on the other hand. Namely, one of the duplicate be acidified to pH 5.5 with 0.1 *N* HCl or acetic acid and kept at 35° C for 15 to 20 minutes; the acid system be then neutralized back to pH 8.5 with 0.1 *N* NaOH and kept again at 35° C for an hour. After the total volume of the neutralized system (v_3 *cc*) is noted down, the mother liquor be estimated of its soluble nitrogen content (μ *mg*) using an aliquot portion (v_4 *cc*) of its filtrate.

The amount of eumyosin nitrogen recovered from the sample will then be computed at $\left(\frac{mv_1}{v_2}-\frac{\mu v_1 v_3}{v_2 v_4}\right)$ *mg*. Hence, eumyosin nitrogen represented as the percentage of total nitrogen of the sample will be given by

$$\frac{100v_1}{Mv_2}\left(m-\mu\frac{v_3}{v_4}\right).$$

Usually, the figure thus obtained does not strictly express the amount of the protein latent in a given sample but are too low by a few per cent or so. Such discrepancy may possibly arise from the incomplete deposition of eumyosin at pH 8.5 (*cf.* foot note 44). The present procedure nevertheless seems of sufficient avail in appraising the relative rate at which a given coagulum of eumyosin degenerates, for instance, during its dry-storage.

V, 3. The Disappearance of Eumyosin During Dry-Storage

As already pointed out (*cf.* HIRANO, 1953, p. 157), the motive which leads eumyosin deposit into a hardly-peptizable mass of xerogel on drying is essentially traceable not to the diminution in water content but to the narrowing of the interstices between the particles constituting the deposit entity. In other words, dehydration is the result but not the cause of the fall in peptizability in this case. Therefore, if water were expelled so rapidly that any appreciable shrinkage is not effected, the resulted mass would retain as excellent a peptization tendency as that shown before drying.

Considered from the fact that the impressions of thixotropy, plasticity, and peptizability exhibited by eumyosin deposit change widely with varying degrees of concentration, it may be inferred that not only the size of elemental particles building up the deposit but the height of potential barriers preventing the particles from intimate coalescence are extremely manifold. Consequently, when a mass of eumyosin shrinks on dehydration, there will prevail an uneven strain throughout the bulk of mass. This strain will, however, be uniformed and relaxed more or less completely sooner or later; and accompaning this, spatial packing of particles will also become more compact, resulting in the withdrawal in peptizability of the mass as a whole. After the packing has developed extremely, whether intensively or extensively, it may be certain that the mass of eumyosin consisting inherently of huge molecules can be no longer peptized without disruption of its constituent molecules themselves.

Then at what rate does eumyosin lose its peptizability during dry storage? And on what factors is dependent the rate of loss?

V, 3, 1. Apparent Durability of Eumyosin in Dry Storage

In order to judge whether it is practicable or not the attempt to preserve raw material for *Kamaboko* as so-called powder of raw fish, it is evidently of primary importance to know the rate in the concrete at which eumyosin is disqualified during dry storage. Table 31 contains an experimental result visualizing the magnitude of the rate in question.

Table 31. Recovery of Eumyosin from its Isoionic Powder Kept over $CaCl_2$. (Stored at room temperature)

Number of sample	Date when a sample was taken out from desiccator	Period of storage (*day*)	Weight of sample (*g*)	Moisture content of sample (%)	In per cent of total N of sample		$\frac{(II)}{(I)} \times 100$
					(I) Extracted N	(II) Eumyosin N	
1	May 26	0	1.000	59.8	99.5	96.3	96.8
2	〃	1/8	0.442	9.1	99.9	96.9	97.0
3	27	1	0.426	5.6	99.2	96.3	97.1
4	28	2	0.418	3.8	100.1	97.0	96.9
5	31	5	0.414	2.9	99.9	97.0	97.1
6	June 4	9	0.408	1.5	99.3	96.0	96.6
7	12	17	0.404	0.5	98.4	95.6	97.1
8	28	33	0.406	1.0	97.8	94.5	96.6
9	July 14	49	0.409	1.7	94.5	89.3	94.5
10	30	65	0.405	0.7	76.9	74.4	96.7

In this experiment, apparently dry material was used which had been obtained by pressing previously-centrifuged isoionic eumyosin against porous plate. Every 1.000 *g* of the material corresponding to 0.402 *g* anhydrous matter or 59.2 *mg* nitrogen, was put in every one of twelve shallow weighing bottles of 20 *cc* capacity. While two of the twelve served the purpose of determining water and nitrogen contents (*cf.* the data given above), the remaining ten were stored at room temperature in a large desiccator charged with $CaCl_2$. Taking out the bottles one by one at different intervals of storage, their contents were estimated of recoverable eumyosin according to the routine procedure (*cf.* V, 2, 4). It should be noticed here, in addition, that the portion left unpeptized also proved to yield a *Kamaboko*-like elastic mass on heating in steam after pressing at the isoionic point. Furthermore, as understood from the data listed in the last column of the table, the stock of eumyosin suffered no intrinsic mutation in *Kamaboko*-forming ability even after it had somewhat receded in recoverability itself : in other words, it receded merely in peptizability. It may be concluded after all that isoionic eumyosin retains its recoverability nearly to perfection even at temperatures prevailing in June to July (about 25 to 30° C in *Tokyo*) for a month or longer if kept desiccated up to the extent of 1% moisture.

V, 3, 2. Diminution of Recoverable Eumyosin during the Storage in Moist Atmosphere

To carry out the present experiment another portion of the apparently dry material employed in V, 3, 1 was thoroughly desiccated beforehand over $CaCl_2$ in vacuum. White substance thus obtained was ground up into fine powder

Table 32. Failure in Recoverability of Eumyosin Kept

Number of sample	Date when a sample was taken out from desiccator	Period of storage (*day*)	Weight of sample (*g*)	Moisture content of sample (%)
1	May 27	0	0.300	5.68
2	28	1	0.341	16.88
3	30	3	0.363	21.9
4	June 4	8	0.363	21.9
5	12	16	0.356	20.5
6	28	32	0.358	20.8
7	July 14	48	0.356	19.6
8	30	64	0.350	19.1

and applied to the preparation of samples to be stored. Every 0.300 *g* of the powder, equivalent to 0.283 *g* anhydrous matter or 41.7 *mg* nitrogen, were put spread into a thin layer in each one of eight shallow weighing bottles. With slipped stoppers these bottles were set in a desiccator having contained saturated solution of sodium carbonate together with $Na_2CO_3 \cdot 10H_2O$ crystals so that the samples were kept exposed to humidity of a definite degree (87%) at room temperature. The bottles were taken out one by one after various periods of storage and weighed in order to estimate the moisture of their contents, after which the amounts of recoverable eumyosin were determined. The results are given in Table 32.

Here again, it was ascertained experimentally that the final insoluble residue, similarly to that in V, 3, 1, exhibited *Kamaboko*-forming ability when strained by pressing at pH 5.5 and heated by steaming.

Comparing the present result with the preceding one (V, 3, 1), it is obvious that dry stock of eumyosin loses the recoverability far more rapidly at a high humidity than at a low.

Now that several facts seem suggested by the present experiment, they will be described separately in the following:

(1) **Moisture content of eumyosin powder.** As seen in the fifth column of Table 32, eumyosin once dried up to a good extent regains moisture promptly on being exposed to humidity. This seems to be accordant with the expectation that the broad variety in the disposition as well as the species of amino acid residues will hold eumyosin molecules in so highly amorphous a state of aggregation that their polar groups stand substantially exposed to the surrounding medium.

The moisture content of the powder at a definite humidity, however, re-

in Moist Air of 87% Humidity at Room Temperature.

In per cent of total N of sample		$\frac{(II)}{(I)} \times 100$	Remarks
(I) Extracted N	(II) Eumyosin N		
100·2	96.0	96.0	Samples appeared white.
99.5	93.7	94.2	Ditto.
99.1	92.9	93.7	White moist powder.
94.9	88.8	93.6	White moist powder; a minute amount of insoluble matter was present.
83.1	75.9	91.3	Tinged with faint reddish yellow; moist powder on the first step of clotting.
58.7	51.2	88.3	Tinged with reddish yellow; in the early stage of clotting.
54.1	44.6	82.5	Reddish yellow; somewhat clotted moist powder.
51.5	39.7	77.1	Ditto.

tires little by little in a long period of storage. This, a sign of spontaneous recession in hydrophilic surface, points to that there develops gradually within particles of the powder some compact regions where some of polar groups on adjacent molecules come hand in hand with one another letting go the water molecules which they have had.

From such a point of view combined with the knowledge on the protein composition, it is inferred that the polar groups which readily adopt or abandon water molecules in response to the fluctuation in humidity may certainly be of ionic nature, while such giving up water slowly but steadily at a definite humidity may probably consist primarily of peptide linkages.

(2) **Apparent sedimentvolume of insoluble residue in a dilute** (0.02 *N* before application) **NaOH solution** varied as shown in the following table:

Number of sample	1	2	3	4	5	6	7	8
Period of storage (*day*)	0	1	3	8	16	32	48	64
Sedimentvolume (*cc*)	0	0	0	2	53	44	40	38

Considered from the fact that the residue lay strongly swollen up (for example, 10–15 *mg* insoluble nitrogen apparently occupied 40–50 *cc* in the alkali solution), the table seems to indicate that in a week or so in the beginning of storage forms practically no insoluble matter even if the moisture content of the powder has attained to an equilibrium (*cf.* the fifth column of Table 32). It will furthermore be evident that while the insoluble matter taking place already in the first week increases steadily if not monotonously as computed from the sixth column of Table 32, its apparent sedimentvolume rises promptly to a maximum

after about two weeks and falls very slowly afterwards, pointing to that extreme bulkiness of incipient residue becomes abolished progressively in the later period of storage. The fact that the swelling ability withdraws not always in parallel with the decreasing peptizability, namely, that the insoluble residue, while it exhibits an enormous sedimentvolume in the early stage of storage despite its minuteness in amount, occupies only a small sedimentvolume towards the end of storage regardless of its far much increased quantity, suggests that the motive causing the decrease in peptizability may certainly be of different nature from the motive effecting the withdrawal in swelling ability. Supposedly, there prevail two kinds of linkages between the particles constituting the mass of eumyosin, the one relatively weak, the other comparatively strong. It seems probable that the observed decrease in peptizability is attributable to the extensive increase of the stronger sort of linkages, while the recession in swelling ability may perhaps be traceable primarily to the multiplication of the weaker sort. What atomic groups are responsible for these two kinds of linkage, respectively, will be discussed later in V, 4.

(3) **Nitrogen remaining dissolved at pH 8.5.** As seen from the difference between the two sets of figures listed respectively in columns 6 and 7 in Table 32, a minute amount of nitrogen ingelatable at pH 8.5 comes extracted together with recovered eumyosin. This ingelatable fraction, however, regardless of its gradual increase towards the end of storage, remains practically constant in amount for a few days in the beginning. So, the fraction may be considered to consist of a part of eumyosin having escaped from gelation but not metaeumyosin at least in the extracts prepared after a few days storage.[44]

(4) **Eumyosin nitrogen.** Considered from the fact that the figures listed in the eighth column, *i.e.*, eumyosin nitrogens represented as the percentage of extracted nitrogens, retire only far much slowly in comparison with those in the columns 6 and 7, the decrease of recoverable eumyosin seems attributable primarily to the difficultly-peptizable-becoming, DPB in abbreviation, of the powder, while the degradation of eumyosin into metaeumyosin makes little contribution to it.

Futhermore, being based on the figures given in the seventh column, if the relative recovery of eumyosin is plotted against the days of storage, a sigmoidal curve will be obtained as illustrated in Figure 22. It will be noticed here that, whereas a portion of the protein becomes impeptizable rather promptly, its remaining portion behaves highly durably, tending to retain the original recovera-

44) For mininizing the amonnt of eumyosin getting out of precipitation, it seems surely of use to decant off the mother liquor at pH 5.5 in order to concentrate the isoionic deposit prior to directly estimating the precipitated nitrogen at pH 8.5. Since, however, a more or less minute quantity of the protein remains dissolved in the condition favorable to induce protonogelation, making it difficult to eject soluble proteinous matters other than eumyosin from the latter, such device to minimize the eumyosin loss will meet with another difficulty in purifying the protein deposit. Under the circumstances, the present procedure is entirely "practical estimation of recoverable eumyosin" but by no means "theoretical estimation of latent eumyosin."

bility almost permanently. What accounts for this state of matters will be discussed later on (*cf.* V, 4).

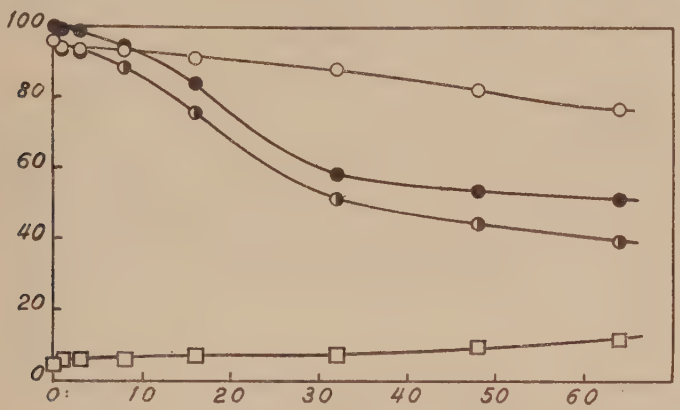

Fig. 22. Relative amounts of various types of nitrogen recovered from a sample of eumyosin powder stored in moist air at 87% humidity at room temperature. Abscissa: Time of storage in *day*. Ordinate: Recovered N in *per cent*. Solid circles: Soluble N as per cent of total N of the powder. Semisolid circles: Eumyosin-N as per cent of the total N. Open circles: Eumyosin-N as per cent of the soluble N. Squares: Non-protonogelatable N as per cent of the total N.

(5) **Discoloration of dry eumyosin in moisture.** As noted down in the last column of Table 32, eumyosin powder which had initially appeared white turned afterwards reddish or brownish yellow in color and clotted into sawdust-like granules. This discoloration developed keeping pace with the decrease in peptizability but not with the increase in moisture content ; and if once developed, it could never be extinguished even though the power was dried up afterwards. Thus, despite that the moisture content of the powder attained to an equilibrium already in a few days during storage, the fall in peptizability as well as the color production became detectable first after about a week, suggesting the prevalence of a time-lag or an induction stage.

Although the origin of the discoloration is as yet uncertain, the phenomenon seems unlikely to be disposed of by merely assuming a simple reaction, say autoxydation of phenolic group of tyrosinyl residue, for intact precipitate of eumyosin scarcely suffers discoloration. It may also be supposed that the discoloration is realized first when some optical structure-sensitive property (*cf.* Makishima, 1952), whether intermolecular or intramolecular, has developed to a substantial extent, due to the protracted distortion of eumyosin molecules.

Anyhow, it is noteworthy that the discoloration is favored by minute contamination of water and further without any appreciable change in the total weight of anhydrous matter (*cf.* Remarks 11). The phenomenon may thus possibly provide a measure for the retirement in recoverability of eumyosin, which proceeds in its dried preparation.

Remarks 9. It was ascertained as follows that the test material used in V, 3, 2 possessed an *explicit Kamaboko*-forming ability :

The dry mass of eumyosin was first ground up into fine powder, while the grinding operation went on quite smoothly, suggesting the high porocity and brittleness of the mass. The powder was then soaked in 0.6*M* potassium phosphate buffer of COHN at pH 7.0[45] and kept for a night in a refrigerator. A little protein was found dissolved in the mother liquor which gave voluminous precipitate when poured into a large amount of plain water, verifying that the protein behaved in accordance with typical myosin. The portion left insoluble, in turn, settled to the bottom only at a considerably small rate when the system was stirred up and allowed to stand subsequently. Moreover, as a matter to be noticed, the insoluble portion was successfully agglomerated into a dumpling-like elastic mass when concentrated by straining ; and the mass turned indubitably into a piece of typical *Kamaboko* when treated in boiling water.

The present experiment offers a fact of extra-importance that it is really possible to prepare such a sort of eumyosin powder as is qualified for *Kamaboko*-formation by mere treatment with neutral salt solution.

Remarks 10. *Kamaboko*-forming ability of impeptizable portion. Another experimental result that even the portion left impeptized after treating eumyosin powder with NaOH solution exhibits *Kamaboko*-forming ability under certain circumstances points to that : Supposedly, even in the particles constituting the residual portion, interfibrillar linkages hindering these particles from dissociation, *i.e.*, dissolution, may be but of small number, due to the inferior tendency of eumyosin fibrils to mutual fitting. The fibrilsegments in the residue therefore may stand so far remote from one another as to behave almost similarly to those in the dissolved portion ; in other words, they may be able to be reagglutinated also between different particles, resulting in a single continuous network needed for the formation of a *Kamaboko*-block, when the residue is once neutralized and concentrated by straining. Whether the residue exhibits *Kamaboko*-forming ability or not on treating in a way as just mentioned seems dependent rather exclusively upon the magnitude of such obstinate intersegmental linkages as remain unresolvable against the present peptizer.

V, 3, 3. The Effect of the Degree of Humidity upon the Recoverability of Eumyosin

In the experiment stated in V, 3, 2, it was experienced that a larger or smaller portion of dry eumyosin remained persistently in such a state as that from which the protein was always ready to be recovered. Then, does it vary with the degree of humidity of the environment and hence with the moisture receipt of dry eumyosin, not only its rate but also the extent of becoming diffi-

45) In preceding two papers related to the present work, the author has committed a mistake in recalculating the molal concentration of COHN's phosphate buffer into ionic strength, having given values as too large as $6 \div 2.40 = 2.5$ times the correct ones. The author has no words to apologize for it, but is now anxious for readers' kindness to correct these errors.

cultly-peptizable? If it does, what is the reason? In order to apprehend the state of affairs involved an experiment was carried out as follows:

As the material to be examined, a powdered preparation of eumyosin was employed which was obtained by promptly drying its isoionic precipitate at a room temperature, while the precipitate was intact one made by neutralizing its NaOH sol.

Every 0.400 *g* of the powder, equivalent to 0.356 *g* anhydrous matter or 52.5 *mg* nitrogen, were put in each one of many shallow weighing bottles of 20 *cc* capacity, which were divided into 3 classes corresponding to the degrees of humidity at which they were stored; namely, (*a*) the storage over calcium chloride, (*b*) the storage at the relative humidity of 75% realized by applying the saturated solution of NaCl, and (*c*) the storage in water added with a few pieces of thymol crystal as anticeptics. These various degrees of humidity were maintained by means of three desiccators kept in an incubator at 40° C, while, as to

Table 33. Recovery of Eumyosin from its Powder Stored at Various Humidities at 40° C.

Number of sample	Period of storage (*day*)	Moisture content of sample	Amount of N extracted with 0.02 *N* cold solution of NaOH (*mg*)			Eumyosin N represented as per cent of	
			total N	N remaining dissolved at pH 8.5	Eumyosin N	total N of sample	the N extracted with the NaOH solution
a: kept over calcium chloride not-calcined. (Relative humidity = 0% (?))							
a 1	0	10.93	52.4	1.52	50.9	97.0	97.0
a 2	1	6.11	52.9	2.33	50.6	96.5	95.7
a 3	7	5.19	50.7	2.10	48.6	92.6	96.0
a 4	15	4.91	39.7	2.53	37.2	70.9	93.7
a 5	24	4.00	36.9	3.12	33.8	64.4	91.6
a 6	30	2.85	36.7	3.53	33.2	63.1	90.5
b: kept over the saturated solution of NaCl. (Relative humidity = 75%)							
b 1	1	13.29	52.1	2.40	49.7	94.7	95.5
b 2	3	15.17	51.4	2.70	48.7	92.9	94.9
b 3	7	14.72	45.2	2.88	42.3	80.5	93.6
b 4	15	15.03	31.9	3.78	28.1	53.5	88.0
b 5	21	14.07	27.4	3.84	23.6	45.0	86.1
b 6	30	14.87	27.3	4.22	23.1	43.6	84.7
c: kept dipped in liquid water. (Relative humidity = 100%)							
c 1	1	—	48.6	3.45	45.1	86.0	93.0
c 2	3	—	38.4	3.25	35.1	66.9	91.5
c 3	5	—	27.6	3.84	23.8	45.3	86.3
c 4	7	—	25.9	4.15	21.7	41.3	83.9
c 5	11	—	26.1	4.97	21.1	40.2	80.9
c 6	15	—	25.8	5.42	20.4	38.8	79.2
c 7	19	—	25.6	5.60	20.0	38.0	78.0

the samples shared to class *c*, their containers, *i.e.*, the weighing bottles, were directly added with every 10 *cc* of water. After different periods of storage, the bottles were taken out one by one and recoverable eumyosin having remained in their contents was determined after the routine procedure. The results are illustrated in Table 33 as well as in Figure 23.

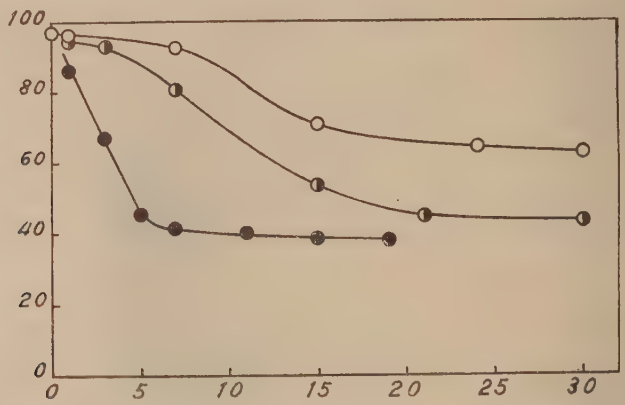

Fig. 23. The loss in recoverable eumyosin during the storage of eumyosin powder in atmospheres at various degrees of humidity at 40° C. Abscissa: Time of strage in *day*. Ordinate: Recovered eumyosin-N represented as per cent of total N. Open circles: Kept over $CaCl_2$. Semisolid circles: Kept over the saturated solution of NaCl (75% humidity). Solid circles: Kept in liquid water.

It will be evident from the present results not only that eumyosin powder becomes impeptizable the more rapidly at the higher humidity but also that the portion becoming impeptizable increases with increasing humidity; nevertheless, some 40% of eumyosin remains recoverable almost for ever even at the saturated humidity and the storage temperature as high as 40° C. Also even the portions left impeptized in this case proved to be more or less capable of *Kamaboko*-formation if they were brought first to pH 5.5, strained next by pressing, and heated finally by steaming, though the residues in (*b*) and (*c*) were found distinctly inferior in *Kamaboko*-forming ability to the residues in class (*a*).

Remarks 11. Because moisture does promote the DPB of eumyosin powder, it is not without reason to suspect *a priori* that the water adopted by the powder might enter into combination with the protein causing the latter to be hydrolysed, for instance, or that the moisture might facilitate the occurrence of some sort of inter- or intra-molecular dehydration such as neutralization between undissociated carboxyl and amino groups or some other sorts of chemical condensation. Such suspicion, however, has been answered to be inferrably of no use by an experiment as follows:

Of the eumyosin powder which was prepared just like that used in V, 3, 2, every 0.500 *g* were put in two shallow weighing bottles of 20 *cc* capacity. The charged bottles weighed previously for estimating their anhydrous matter contents by drying up over P_2O_5 in vacuum, were kept exposed to humidity (75%) in a

desiccator having contained saturated solution and saturating body of NaCl under maintaining the storage temperature at 40° C. In a prolonged storage during which the bottles were weighed from time to time the samples became discolored so as to appear strongly reddish yellow but remained at a definite moisture content. The detail of the experiment is given in the following :

The weighing bottles employed were as follows : No. 290=12.6619 *g* ; No. 297=12.7252 *g*.

Weight of the bottles charged with powder.

Date	No. 290	No. 297	Remarks
Aug., 23	13.1619 *g*	13.2252 *g*	Immediately after the charge of powder.
24	13.1149	13.1749	After kept over P_2O_5 in vacuum for various periods.
25	13.1133	13.1742	
26	13.1134	13.1742	

From this table which points to that the moisture content of the samples attained to an equilibrium in first two days, the unvolatile matters contained in the bottles were estimated at 0.4514 *g* for No. 290 and 0.4490 *g* for No. 297, respectively. (In the present case sampling operation proved to be accompanied by a fluctuation attaining to $100(0.4514-0.4490)/\frac{1}{2}(0.4514+0.4490)=0.5\%$.)

Weight of the charged bottles kept over saturated solution of NaCl at 40° C.

Date	No. 290	No. 297
Aug., 27	13.1805 *g*	13.2400 *g*
28	13.1811	13.2409
29	13.1809	13.2406
30	13.1809	13.2407

As seen from these data the samples weighed 0.5190 *g* for No. 290 and 0.5155 *g* for No. 297, respectively ; and hence their gains in weight due to moisture sorption, represented as the percentage of dry matter, are computed at 15.0% for No. 290 and 14.8% for No. 297. (The deviation from one another attained to 1.3%.)

Weight of the bottles kept further over the NaCl solution.

Date	No. 290	No. 297	Remarks
Sept., 7	13.1803 *g*	13.2405 *g*	The samples became colored strongly reddish yellow in this period.
23	13.1803	13.2404	

Thus, in despite of their notable discoloration, the samples underwent but slight decreases in weight since August 30.

Weight of the bottles brought back over P_2O_5 in vacuum.

Date	No. 290	No. 297	Remarks
Oct., 14	13.1178 *g*	13.1783 *g*	
20	13.1141	13.1744	
21	13.1134	13.1743	
23	13.1133	13.1741	
25	13.1132	13.1739	
27	13.1134	13.1740	The samples remained strongly colored.

From the above data, the amounts of unvolatile matter were estimated at 0.4515 *g* for No. 290 and 0.4492 *g* for No. 297, quite in accordance with those before storage.

It follows from the present observations that eumyosin powder, even if strongly discolored in humidity, neither combines nor sets free any volatile substance to a measurable extent.

Since this result means that the dipole of water can reversibly come into or go out of linking with eumyosin, the same leads further to a notable suggestion that any possible sorption taking place between the protein and a dipole, whatever polar group on the protein molecule may constitute the partner for the latter, would certainly be of reversible nature at least so far as the dipole is of small molecule. Therefore, if the apparently-irreversible coagulation of eumyosin is to be attributed to the intermolecular coherence effected by the mutual attraction of polar loci on the protein molecule, *e.g.*, hydrogen-bond-formable peptide groups, it should be concluded that the said irreversibility might certainly be but virtual and ascribable solely to the extraordinary hugeness of eumyosin molecule. Such a point of view seems to be supported by the fact that eumyosin coagulum which behaves as quite an insoluble matter in a weak solvent generally allows its larger or smaller fraction to be dissolved if a sufficiently strong solvent is applied to it.

V, 3, 4. The Influence of Initial pH upon the Tenability of Dry Eumyosin

As already mentioned, the extent to which an eumyosin deposit loses its peptizability in neutral salt solution on being dehydrated depends upon the pH prevailing in the deposit immediately before dehydration. From here it is expected that the rate of DPB of dry eumyosin also depends upon the pH directly prior to drying. This expectation has been verified on the basis of the experiment of which result is illustrated in Table 34 as well as in Figure 24.

In this experiment five subdivisions of an eumyosin deposit washed thoroughly with distilled water were first differentiated in pH by adding various amounts of NaOH or HCl solution. They were then concentrated by centrifuging, straining with porous plates, and desiccating over P_2O_5 under reduced pressure. The dry matters thus obtained were ground up into fine powder, respec-

tively, and used as samples as follows: Every 0.400 *g* of each one of these five different powders were placed spread in every one of five shallow weighing bottles of 20 *cc* capacity. Five sets of samples composed of twenty five individuals were thus provided in the total. All these samples with unfastened caps of their containers were then put together under a large glass dish set face down over them and kept exposed to the temperature and humidity of laboratory. The amounts of eumyosin recoverable from these samples were estimated by the routine procedure at various intervals of storage.

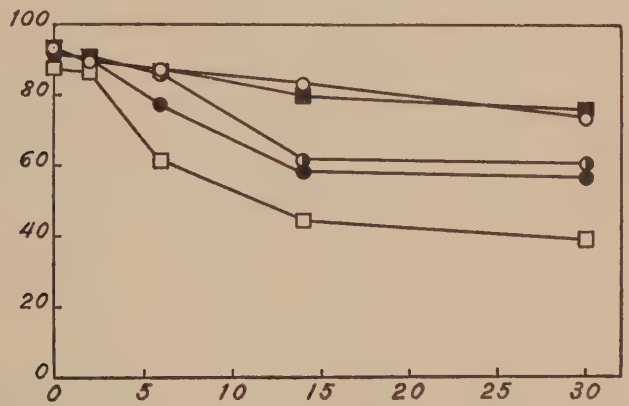

Fig. 24. Spontaneous loss in recoverable eumyosin in different eumyosin powders prepared starting from various pH's. Abscissa: Time of storage in *day* (at the temperature and humidity of laboratory). Ordinate: Recovered eumyosin-N represented as per cent of total N. Open squares: From pH 9.0. Solid squares: From pH 7.0. Open circles: From pH 5.5. Semisolid circles: From pH 4.5. Solid circles: From pH 4.0.

It is obvious that, from Figure 24 in which the results are shown, dry eumyosin is tenable most safely if it has been prepared from the raw material in the neighborhood of, or strictly speaking, in a narrow pH range slightly basic to, the isoionic point of eumyosin.[46]

46) Inferred from the dissociation constants of ionogenic groups expectable in correspondence to the constituent amino acids, eumyosin is likely to acquire most abundant pairs of oppositely-signed electric charges at a pH between 6 to 7, though the protein becomes isoionic at pH 5.5. It is also not inconceivable in the case of highly concentrated eumyosin that the dissociation of carboxyl groups is suppressed more intensely than that of ammonium groups, shifting the point of minimum net charge to a reaction more basic to the isoionic point which has normally been defined for a dilute system.

Table 34. Tenability of Various Preparations of Eumyosin Powder Obtained temperature and humidity of laboratory.

Number of sample	Period of storage (*day*)	Weight of sample (*g*)	Moisture content of sample (%)	Composition of N extracted with 0.02 *N* cold solution of NaOH		
				total (*mg*)	meta-eumyosin (*mg*)	eumyosin (%)
						Series I: Drying
I-1	0	0.403	5.3	53.2	4.5	48.7
I-2	2	0.442	14.8	51.5	3.4	48.1
I-3	6	0.442	14.8	39.4	5.1	34.3
I-4	14	0.441	14.7	30.4	5.6	24.8
I-5	30	0.439	14.1	26.5	4.8	21.7
						Series II: Drying
II-1	0	0.402	5.0	54.6	2.8	51.8
II-2	2	0.438	14.1	53.2	2.7	50.5
II-3	6	0.437	14.0	52.6	4.2	48.4
II-4	14	0.438	14.1	51.8	7.5	44.3
II-5	30	0.436	13.7	47.5	5.3	42.2
						Series III: Drying
III-1	0	0.400	4.7	55.5	3.6	51.9
III-2	2	0.437	14.0	53.7	4.0	49.7
III-3	6	0.435	13.6	52.6	4.8	48.4
III-4	14	0.429	12.2	52.8	6.7	46.1
III-5	30	0.433	13.1	46.5	5.9	40.6
						Series IV: Drying
IV-1	0	0.400	4.5	55.1	3.5	51.6
IV-2	2	0.434	13.2	53.7	3.3	50.4
IV-3	6	0.433	13.0	53.8	6.0	47.8
IV-4	14	0.432	12.9	41.6	7.3	34.3
IV-5	30	0.431	12.7	39.8	6.2	33.6
						Series V: Drying
V-1	0	0.394	3.4	53.7	2.8	50.9
V-2	2	0.434	13.2	53.7	3.2	50.5
V-3	6	0.433	13.1	49.5	6.4	43.1
V-4	14	0.431	12.7	40.1	7.7	32.4
V-5	30	0.431	12.7	39.4	8.0	31.4

by Drying the Protein Deposit at Different pH's. (Samples were kept at the (July ty August, 1943; in *Tokyo*.))

Recovery amount of eumyosin N in per cent of		Remarks on the insoluble residue in the NaOH solution
total N of sample	initial recovery	
started from pH 9.0		
87.8	100	Trace in amount.
86.7	98.7	Sedimentvolume=30 *cc*.
61.7	70.2	Sedimentvolume=64 *cc*.
44.7	50.9	Slightly yellow; sedimentvolume=47 *cc*; (clear mother liquor.)
39.1	44.5	Sedimentvolume=36 *cc*.
started from pH 7.0		
93.4	100	Trace in amount.
91.0	97.5	Ditto.
87.2	93.4	Ditto.
79.9	85.5	Yellowish in appearance; sedimentvolume=5 *cc*; (Hazy mother liquor.)
76.1	81.5	Sedimentvolume=42 *cc*.
started from pH 5.5		
93.6	100	Entirely dissolved.
89.6	95.7	Practical complete dissolution.
87.2	93.1	Ditto.
83.1	88.8	Practically white or colorless; trace in amount. (Slightly hazy mother liquor.)
73.2	78.2	Sedimentvolume=41 *cc*.
started from pH 4.5		
93.0	100	Entirely dissolved.
90.7	97.5	Trace in amount.
86.1	92.6	Sedimentvolume=41 *cc*.
61.8	66.5	Strongly yellowish; sedimentvolume=48 *cc*. (Slightly hazy mother liquor.)
60.6	65.2	Sedimentvolume=46 *cc*.
started from pH 4.0		
91.7	100	Entirely dissolved.
91.0	99.2	Trace in amount.
77.7	84.7	Sedimentvolume=8 *cc*.
58.4	64.1	Yellowish; sedimentvolume=38 *cc*. (Hazy mother liquor.)
56.6	61.8	Sedimentvolume=52 *cc*.

V, 4. Discussions on the Mechanism of the Withdrawal in the Recoverability of Eumyosin during Dry Storage

On the basis of the experimental results described in V, 3, it may positively be concluded that dry eumyosin retains its recoverable *Kamaboko*-forming ability most perfectly when it has been prepared by promptly dehydrating the isoionized deposit at a low temperature and the dehydration product is stored at a humidity and temperature as low as possible. However, securing of low temperatures and humidities proves frequently to be a matter of trouble ; though it may provide a sufficient condition, it seems not always to be the necessary factor to minimize the DPB of eumyosin during dry storage. It appears furthermore not to be too simple in the technical point of its performance and hence to be accompanied by some practical difficulties in regard of that popular *Kamaboko* should be a commodity of easy purchase. Hereon, there become naturally given rise to thoughts that it would be a great convenience to have such powder usable for *Kamaboko*-preparation as is tenable just alike to normal flour even when kept merely in cloth-or paper-bags in a room without any special air-conditioner.

Surely, it will be quite reasonable to conceive that spontaneous DPB of eumyosin is due to the formation of hardly-resolvable linkages among colloidal particles of the protein. But, among what sorts of atoms or groups and in what manner are such linkages given rise to?

If we can seize the proper answer to this problem, we will be able to appoint the not only necessary but also sufficient condition to prevent the formation of such interparticlar linkages taking advantage of the characteristics of the atoms or groups involved even without appealing vulgarly to low-temperature and-humidity ; and thus it will also be able to predict as to whether the appointed condition will feasibly be fulfilled or not. In this section, hereupon, it is intended to draw an inference about the nature and the origin of the linkages in question from the discussion on the fundamentals underlying the experimental results already mentioned in the light of colloid chemistry of protein.

As shown in V, 3, 4, powders prepared by drying eumyosin precipitate at different pH's behave also differently in their tenability from each other even at a definite temperature and humidity, pointing to the significance of protolytic groups on the protein molecules. On the other hand, however, any eumyosin powder undergoes DPB more or less readily whatever pH the protein has had before drying. It may be inferred therefore that the motive inducing the DPB is exerted by such atoms or groups as are little influenced by the pH of environment, while the protolytic groups serve rather as checks restricting the protein particles from being intimately agglutinated by the action of the former. Then, what is the origin of this coalescent force? To what state of aggregation does this force lead the particles? And in what manner is controlled their aggregation?

Considered from the appearance that the controlling effect exhibited by the protolytic groups is slackened with increasing humidity of environment, *i.e.*, with increasing moisture up-take of the protein powder, it may be inferred that

these groups exist in dry powder forming a barrier of salt-bridge enclosing each individual particle or building a set of props of salt-bridge between the interacting particles so that the atoms or groups under consideration are prevented from coming into contact with one another. Then, do there exist such protolytic groups actually on the molecule of eumyosin as are capable of forming such salt-bridge? Yes, there may be. For, as already mentioned (*cf.* HIRANO, 1953, p. 118), above 1/3 of constitutent amino acid residues of eumyosin are inferrable to consist of protolytic ones carrying COO^-, NH_3^+, or others, while these groups are presumedly disposed more or less distantly outwards from the body of molecular chain (*cf.* HIRANO, 1953, p. 160). Then, are there also present such atoms or atomic groups within the protein molecule as exert the coalescent forces upon the protein particles independent of external ionic condition? Yes, they are also present. The peptide linkages, -NH·CO·····NH·CO-, constituting a major portion of the helical chain are correspondent to them.

Since peptide linkages possess a remarkable tendency to mutual formation of hydrogen-bonds (*cf.* PAULING, 1940), such large molecules as those of eumyosin which contain numerous peptide linkages at frequent and orderly intervals are expected to exert polar VAN DER WAALS-LONDON forces of long range upon each other. In the neighborhood of the isoionic point, however, when two or more molecules come very near to one another, there will be formed in the first place only salt-bridges among them by reason of that the protolytic groups stand quite externally to the peptide linkages as to their situation on the protein molecule. Consequently, in fresh precipitate or its dry product, almost all the linkages prevailing between eumyosin particles are presumed to be salt-bridges. Under such circumstances, interparticlar intimate contact due to LONDON-VAN DER WAALS forces are prevented substantially if not perfectly by preceding formation of salt-bridge.

But, if such salt-bridges are loosend by chance or artificially, the particles are expected to come into contact by LONDON-VAN DER WAALS forces with one another so as to be linked together by hydrogen-bondings.[47] However, it must

47) For instance, a fresh deposit of eumyosin formed at the bottom of the water *readily* yields a highly sticky paste if concentrated previously by straining so that the distance between the protein particles is reduced sufficiently and then mixed with NaCl so that prevailing interparticlar salt-bridges are weakened as the result of the formation of soluble amphisalt between NaCl and the protein, corresponding to an instantaneous peptization characteristic to the ionic reaction. The paste solidifies *gradually*, however, while kept quietly, into a mass of jelly as interparticlar hydrogen-bonds are newly developed due to LONDON-VAN DER WAALS forces still prevailing. These two reactions, loosening of salt-bridge and strengthening of hydrogen-bond, constitute a major portion of the essentials of *Kamaboko*-jelly formation phenomenon, though the latter requires furthermore an additional condition that the particles involved should be of fibrous configuration.

It may be possible, according to the comprehensive discussion upon colloidal phenomena performed by HAMAKER (1936, 1936), that even a lyophilized system of colloid behaves thixotropically if concentrated appropriately. Such tendency was frequently observed also with concentrated sols of eumyosin. In the routine process of *Kamaboko*-manufacture, the concentration of myosin in raw material is kept high by straining-off the excessive water prior to the addition of common salt as lyophilizer. In such a point of view, *Kamaboko*-jelly may well be regarded as a sort of saturating body in NaCl-solution, which is standing on the way to complete crystallization.

be kept in mind that on the molecule of eumyosin, these protolytic groups and peptide linkages are disposed so closely to one another that the steric hindrance between them comes often into question. In such circumstances, it is expected not only that the chance by which the intermolecular contacts by these numerous groups are established with one accord is vanishingly small but also that antecedent contacts influence the subsequent ones in one case promotively but in the other case preventively. In the following, it will be described as to how the abovementioned view is supported of its validity by experimental results.

V, 4, 1. Sigmoidal Procession of DPB

As shown by experimental results given above (*cf.* especially Figures 22 and 23), the rate at which DPB develops in process of time remains insignificant in the beginning, grows more or less conspicuous about the middle, and becomes again vanishingly small towards the end, describing a sigmoidal curve. Moreover, the final extent of DPB as a rule does not attain to 100% even when the protein powder is kept in liquid water. In other words, the procession of DPB, while promoted autocatalitically in the early stages, is rather hindered finally by the preceding DPB automatically. This impression of sigmoidal procession strongly supports the abovementioned view upon the colloid chemical mechanism of DPB for the reason explained below.

Incipient autocatalytic development of DPB. The slackening of one of the two forces which have been balanced against each other, *i.e.*, the weakening of the retraction due to salt-bridges, induces a state of matters in which adjacent molecular chains are drawn strongly to one another by means of LONDON-VAN DER WAALS forces. In fact, however, in order that peptide linkages belonging respectively to separate molecules come into contact with one another between the latter so intimately as to contribute to the DPB of the protein mass, the side chains of the protein molecule, including those protolytic groups just released from salt-bridge, must change their posture on the molecule so as not to obstruct the exposure of peptide linkages. Moreover, since it is expected that, upon mutual contact of such peptide linkages, they are bundled together by units of segment consisting of a number of amino acid residues (*cf.* the discussion upon the nature of knots within the network structure of protein xerogel mentioned on p. 66), the chance by which these side chains assume new postures with one accord will be vanishingly small. And hence, the initial concentration of such segments in a fresh mass of protein as are able to enter into contact with one another is also expectable to be negligibly low. The experimentally observed extreme slowness of incipient development of DPB well endorses such expectation.

However, if any pair of such fibrilsegments come into contact with one another by chance, this will necessarily be followed by mutual approach of further number of segments contiguous to the initial pair so that the following pairs of segments come also into contact with each other, because the reciprocal potential energy between these interacting segments will substantially be diminished on their mutual contact in alignment (as inferred from the information by DE BOER (1936)). Such a state of matters will obviously result in autocatalytic development of coherent regions among the fibrils constituting a mass of eumyo-

sin. This view on the mechanism of initial development of DPB seems perfectly supported by the experimental results obtained in V, 3.

Final limited growth of DPB. As will be concluded later on (*cf.* p. 66), it seems likely that in a dry mass of eumyosin, which has suffered DPB to a slight extent, each one of numerous fibrillar micelles runs passing through randomly directed bundled regions here and there which are built-up by the participation of some other fibrils, while majority of the fibrils partaining to different bundles are also different from bundle to bundle (*cf.* Figure 25a). The volume contraction observable on concentrating an eumyosin gel seems interpretable satisfactorily

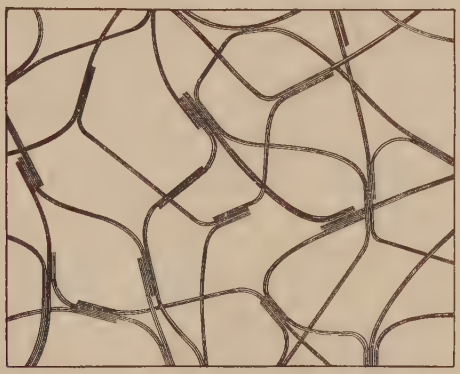

Fig. 25a. Two dimensional schematic representation of the network structure of an eumyosin mass, showing here and there the bundled regions of fibrils.

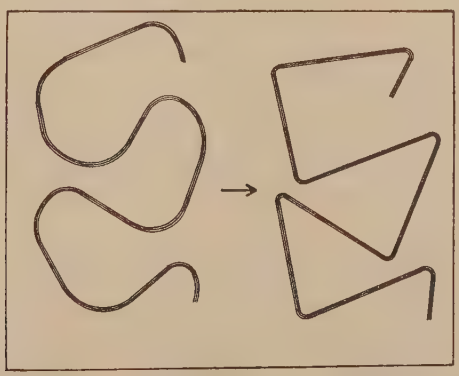

Fig. 25b. Showing the occurrence of abrupt turnings with the development of staightened portions on a fibril.

by the development of such bundled regions as just conceived, though the degree of molecular orientation in these regions will remain but imperfect, for, any excellent intermolecular fitting cannot be expected of course from the molecular structure of eumyosin (*cf.* Hirano 1953, p. 160).

As readily presumed from Figure 25a, each fibril in the mass is conditioned so as to assume variant and only-restrictedly-changeable directions in its several portions partaking different bundles. Under these circumstances, if the bundled regions enlarge themselves without abolishing the said requisite, there will be given rise to a transition in fibril configuration from roundish to angulated (*cf.* Figure 25b). Consequently, with the advance of such transition, *i.e.*, with the enlargement of straightened portions within one and the same fibril, the latter will be forced to alter its direction the more abruptly in those loci where it enters into or comes out of bundled regions. On the other hand, however, such abrupt change in fibril direction, *i.e.*, extreme bending of micelle, will obviously be opposed by its repulsion due to rigidity. Hence, the framework system of the mass attains eventually to a state of balance wherein the growth of bundled regions becomes arrested halfway. This situation seems corresponding to nothing but the limited growth of DPB which was observed experimentally towards the end of the storage of eumyosin powder.[48]

V, 4, 2. Dependency of Final Extent of DPB upon Humidity

Since the moisture absorbed into a dry mass of eumyosin will act so as to loosen its intermolecular polar linkages, mutual slippage of the molecules within a fibrous micelle will be facilitated the more completely, the higher the humidity of environment. This means that eumyosin fibrils become less rigid or more ready to be bended ; so, it may well be expected that bundled regions, and hence DPB, can develop to the higher extent at the higher humidity if other factors remain unaltered. The results of experiment appear to be quite in accord with such expectation.

V, 4, 3. Dependency of DPB of Eumyosin Powder upon the pH of Raw Material

At first sight, it seems quite incomprehensible that an eumyosin mass retains its original peptizability most safely at the isoelectric point, for, the protein is flocculated most readily at the isoelectric point, while it has been taught that increased tendency to flocculation of protein frequently constitutes an indication of its denaturation. However, on the basis of the view on the colloidchemical mechanism of DPB arrived at through the present investigation this situation is readily understood as follows :

As already mentioned (*cf.* HIRANO, 1953, p. 162), it is expected not only that eumyosin is furnished with equal quantities of positive-and negative-charge but also that its total charge, *i.e.*, the sum of these mutually-oppositely-signed charges, attains to a considerable amount at its isoionic point, though the total charge seems in fact to attain to a maximum at a certain reaction situated between the isoionic point and the neutrality. Hence, in a dry mass of eumyosin prepared from the isoionic deposit, intermolecular salt-bridge will be formed most

48) This view leads further to an expectation that such a dry mass of eumyosin as behaves more inaccessibly to DPB will perhaps be attained when a large number of short bundled regions have formed throughout the original deposit of the protein compared with the case when a small number of long bundled regions have come into being.

frequently so that the induction period of DPB is prolonged to the utmost by the mechanism already explained. The experimental results are found quite in agreement with this expectation.

Moreover, it is also presumable, according to MEYER's suggestion (1929), that helical chains of eumyosin molecule assume at the isoionic point an extremely contracted, and hence, thickest configuration as the result of maximum development of intramolecular salt-bridge. This will cause the protein fibrils to be increased substantially in their resistance against bending so that their alignment into bundled knots in a framework, which is directly responsible for the DPB of an eumyosin mass, is suppressed materially. These discussions postulating the minimum DPB of isoionic eumyosin powder are also entirely approved by the experimental results given in V, 3, 4.

Remarks 12. On the basis of DEBYE-SCHERRER *X*-ray photographs taken for two preparations of eumyosin which behaved quite differently from each other in respect of peptizability, two microphotometer curves were drawn as illustrated in Figure 26.[49] In the figure are shown those obtained by faithfully tracing the original photographs simultaneously in superposition so as to be precisely compared

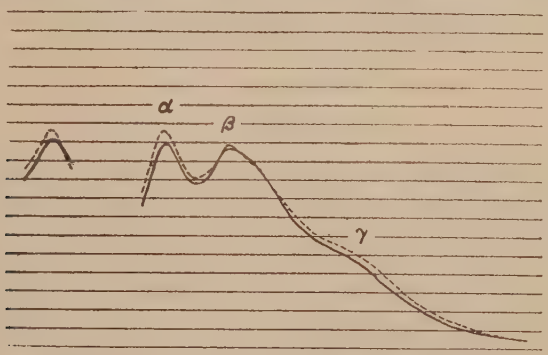

Fig. 26. Microphotometer curves of *X*-ray diffraction traced for two differently aged eumyosin powders, shown in superposition. Solid line: For a fresh preparation. Broken line: For an aged powder having suffered a substantial loss in recoverable eumyosin content.

with one another. Sample *A* dried up in vacuum had been kept sealed in a glass tube for about half a month prior to photographing; it appeared white, not only swelled but dissolved appreciably in NaCl solution, possessing such a naked *Kamaboko*-forming ability as operated on the direct treatment with 0.6 *M* potassium phosphate (*cf.* Remarks 9). Sample *B*, on the other hand, was prepared about eleven years ago and had been kept stored since that time at room temperature in a bottle without any special seal. It was colored lightly reddish yellow; and, in spite of that it had initially an immediate *Kamaboko*-forming ability comparable to that of sample *A*, it behaved virtually inaccessibly to the phosphate

49) The photographs were prepared by courtesy of Professor S. MIYAKE and Mr. K. SUZUKI of *Tokyo Institute of Technology*, for which the author tenders his thanks heartily.

buffer and left a considerable residue swollen but undissolved even in 0.01 N NaOH.

As seen in Figure 26, slight but distinct variations are discernible between these two curves : three protuberances assigned as α, β, and γ, respectively, are more pronounced on the curve for sample A than on that for sample B not only in their height but also in their sharpness. These situations point altogether to that the regions consisting of oriented atomic groups, whether intermolecular or intramolecular, become developed within the particles of eumyosin powder in a long duration of storage.

Apart from the supposition that such orientation of atomic groups may perhaps be established by the intersegmental alignment of peptide linkages, it is truly remarkable that the development of the oriented regions is but of slightness and yet sufficiently effective to reduce the peptizablility of the powder substantially.

Since the abovementioned view on the mechanism of the development of DPB is wholly supported by the experimental results obtained, including the information given by the X-ray diffraction photographs (*cf.* Remarks 12), it may be concluded that within the knots of the network constructing a mass of eumyosin, the protein fibrils lie *not intersectingly* but *in parallel to* one another.

ADDENDUM

Recently, reading BAILEY's paper upon *tropomyosin* of rabbit [*Biochem. J.*, **43**, 271 (1948)], I found that the protein fraction agreed in various respects with my *pseudomyosin* of turbot, supporting BAILEY's view postulating tropomyosin to be an inherent substance to myofibril. The said paper of BAILEY, however, had been yet unable to be gotten in Japan before my description about pseudomyosin, which could not be published till 1951 despite its having been ready for announcement in 1942 (*cf.* foot note 8) in HIRANO, *J. Chem. Soc. Japan*, **63**, 1083 (1942)).

VI. CONCLUSION

Before the author was determined to devote himself to the study for the preparation of *powder of raw fish*, he first inquired experimentally into the suspicion, whether or not fish flesh could be dried actually without losing its *Kamaboko*-forming ability, for the subject of such powder had been left those times entirely within the reagion of a day-dream. The result arrived at was as follows: Fish flesh is really possible to be dried up into such a preparation as is qualified to be employed satisfactorily as the raw material for knead products resembling sausage, such as *Chikuwa*, *Kamaboko*, and etc., at least soon after the preparation. The only key to success in this case was that: the desiccation should de performed rapidly at a low temperature. For example, a small amount of fish flesh previously crushed thoroughly, spread upon wire gauze over the P_2O_5 or concentrated H_2SO_4 in a desiccator kept dipped in an ice-bath, and dried promptly by evacuation provided frequently if not invariably[50] a satisfactory product which was manufactured into *Kamaboko*-jelly after the routine procedure applicable to normal raw fish. This encouraged the present author so that he engaged next in larger-scale preparation of powder of raw fish by means of a spray dryer, a centrifugal atomizer, at Dr. TAMARU'S recommendation.

Many difficulties encountered on carrying out the TAMARU'S plan as it was need not be restated here (*cf.* HIRANO, 1951, p. 204); but the present author wishes to point out that reported *Kamaboko*-forming ability of the fish powder prepared by him with the said dryer should have been of misjudgement, for it has proved certain afterwards that eumyosin, the unique *Kamaboko*-forming entity of fish flesh, should not have existed practically in such a preparation. Anyhow, the author is now obliged to recognize that globulin-*X*, which is extractable with a highly diluted salt-solution from crushed fish at highest freshness but strongly inclines to becoming insoluble in salt solutions, would have certainly been responsible for the quasi-*Kamaboko*-forming ability of the reported powder of raw fish.

In Japanese patent No. 76628 (TAMARU, 1928), it has been expected that raw fish will be dried up into *Kamaboko*-formable powder if its dehydration is accomplished very rapidly at a sufficiently low temperature but avoiding its freezing. The author is really struck with admiration for late Dr. TAMARU'S discernment having pointed to that performing the dehydration at a low temperature and a high velocity was the key for the problem. Since, however, no investigator had ever attacked the phenomenon of *Kamaboko*-formation before that time

50) It should be noticed that in spite of that eumyosin is invariably dried up into a *Kamaboko*-formable product in so far as the operation is carried out justly, it is not always the case with fish flesh as it is. In the latter myosin fibrils are arranged in excellent orientation among each other contrary to the case of eumyosin precipitated from its solution. Such distinction in fibril alignment seems not entirely indifferent to the result of drying. Moreover, the influence of *rigor mortis* which is known in reducing the extractability of myosin with salt solutions may also constitute an important factor in drying intact fish flesh.

it remained quite obscure that, through what reaction of what substance are the phenomenon brought about. In the circumstances, one factor of essential importance had been overlooked in the TAMARU's plan. Thus, it had been unawared that *Kamaboko*-formable entity of fish flesh, whereas it can yield *Kamaboko* only in such a fine degree of dispersion as that realized by employing a spray dryer, could hardly be brought into a fluid suitable to be sprayed. But as it was manifested before long that the said entity was myosin known to be insoluble in plain water, another method was called for whereby raw fish can be pulverized without loss of *Kamaboko*-forming ability if not liquefied beforehand. However, charming procedures such as the xerogel preparation carried out by KISTLER (1932) or the lyophile dehydration applied by FLOSDORF and MUDD (1935) to serum were criticized to be quite unpractical for the preparation of so cheap a commodity like *Kamaboko*. The procedure adopted at last was the plan registered as Japanese Patent No. 158070 (HIRANO, 1943) in which the following scope of privilage was asserted :

Raw fish flesh crushed previously is brought first to the neighborhood of the isoelectric point of myosin by treating the flesh in a dilute solution of acid or acid-salt, strained next from water by pressing, and dried up finally at a low temperature.

In this patent-script the author applies to fish flesh his experience that eumyosin is desiccated and also preserved with least DPB when the protein has been in isoionic state. In applying the product to the preparation of *Kamaboko*, the former is first peptized in a cold solution of NaOH at a pH of about 12 ; then whole the system is neutralized with HCl down to a pH of about 6 ; and the precipitate formed then, together with originally insoluble matter, is strained off from excessive water, after which the pressed residue is processed just like ordinary bleached fish.

SUMMARY

The most significant attainment in the present work consists in the discovery of the two specialities pertaining to *Kamaboko*-forming protein fraction of fish flesh : (1) the second maximum of flocculation at pH 8.5, a reaction quite different from the isoelectric point, and (2) the highest peptizability shown by the isoelectric precipitate. On the basis of the first characteristic, the author has succeeded in both isolation and estimation of the *Kamaboko*-forming entity with great ease. Taking advantage of the second speciality, he has been able to prepare dry fish being usable as the raw material for *Kamaboko*. The investigation made about the origin of these specialities has enabled the author to interpret satisfactorily the nature of *Kamaboko*-formation phenomenon which has so far remained yet inexplicable. The affairs dealt with in this connection will be summarized with some complementaty statements as follows :

1. In order to search the origin of *Kamaboko*-forming ability of myosin from various angles, an improved procedure for the isolation of the protein fraction has been put forward, after which it has been made possible to prepare a large

amount of the essence of *Kamaboko* with great ease and at a small expense.

Employing freshly minced flesh of turbot the following facts have been ascertained experimentally :

1, *a*. When the minced flesh is washed thoroughly with 0.02 *M* sodium-acetate-acetic acid buffer at pH 7.0, bleached fish completely free from soluble proteins except myosin is obtained without insuring the latter at least in respect of *Kamaboko*-forming ability.

1, *b*. Even when bleached fish contains denaturation products of myogen or globulin-*X* already deprived of inherent solubility, it is possible to precipitate myosin alone in a practically pure state if we first extract the bleached fish with a cold dilute solution of NaOH, then acidify the extracted liquor to pH 5.5 by adding HCl, and finally bring the acid system back to pH 8.5 by the addition of NaOH.

2. Myosin coming extracted from bleached fish in NaOH-solution has proved to consist at least of two fractions. The one fraction possessing *Kamaboko*-forming ability has been named eumyosin, while the other lacking the ability has been designated as pseudomyosin.

3. It has been assumed that eumyosin is freed from pseudomyosin by repeatedly washing the myosin precipitate with an extremely-diluted solution of NaOH at pH 8.5 until a stational state is attained in which the amount of protein coming extracted does not decrease any further with increasing number of times of washing. Finally the NaOH contamination is ejected by thoroughly washing with plain water.

4. Of pseudomyosin several properties besides *Kamaboko*-forming ability have been observed or estimated, such as, flowbirefringence, spinning ability, solubility and viscosity at various pH's, dispersibility in NaCl-solutions, salting out with $(NH_4)_2SO_4$, acid and base combining capacities, coagulation by heat, *etc*. Considering the result after referring to earlier works of foreign investigators it has been concluded that pseudomyosin may be regarded as an innate protein fraction of cross-striated muscle of fish, a component of classical myosin devoid of *Kamaboko*-forming ability.

5. Using purified preparations of eumyosin have been carried out observations or measurements as follows which seem necessary for comprehending the nature of *Kamaboko*-formation phenomenon.

5, *a*. Inquiring into the effect of pH upon the solubility and viscosity in HCl- or NaOH-solutions it has been ascertained that the protein is entirely insoluble at any pH between 5 and 7. Its precipitate, excepting that just made, is less soluble in HCl-solutions than in NaOH-solutions. Intrinsic viscosity of eumyosin attains to a maximum at a pH of about 8.5 where its visional sol at 1% concentration would exhibit as high a viscosity as 10 to 20 times that of water.

5, *b*. By means of PAULI's apparatus the isoelectric point of eumyosin has been determined electrophoretically to be situated at pH 5.5 in close accordance with published data for classical myosin.

5, *c*. On the basis of the appearance, *i.e*., the turbidity, the liquid-elasticity, and the viscosity, as well as the limit in restoration of flowbire-

fringence of eumyosin sols in HCl-or NaOH-solutions, especially in the latter, micelle formation of eumyosin has been suggested.

5, *d*. Taking into account of the electrolytic dissociation of ionogenic groups belonging to the constituent amino acid residues, it has been discussed the reason why the solubility curve of freshly precipitated eumyosin does not run symmetrically around the isoionic point.

5, *e*. From the measurement of pH, titrating eumyosin suspensions with varying amounts of HCl or NaOH, the equivalent weight of the protein as an acid or a base has been computed at 300 or 400, respectively, far much small values compared with those of classical myosin ever published. The present result suggests that about 1/3 of amino acid residues constituting an eumyosin molecule are of protolytic nature.

5, *f*. The solubility of eumyosin in NaCl solutions at pH 7.0 containing 0.05 *M* phosphate buffer of COHN has been found to vary with changing salt concentration in close resemblance to that of classical myosin. In spite of its stability against the gravitational force, NaCl-eumyosin sol does not entirely get through the pores of filter paper gradually clogging them during filtration. This suggests simultaneously the hydrophlicity and the fibrousness of dispersed particles.

It has also been pointed out that the solubility in NaCl-solutions is sharply dependent upon the pH of the latter, namely, the dissolving power of NaCl is markedly suppressed by minute coexistence of acid or base. In this case the protein is most effectively salted-in at a pH around 6. The situation has been discussed from the change in dipole moment effected by one-sided ionization of the protein.

5, *g*. The viscosity of an eumyosin sol containing NaOH decreases as a rule more or less gradually with the lapse of time following the course of a first order reaction, But in an extremely dilute solution of NaOH containing the protein at an extremely high concentration, it often rises up to as large a value as nearly 100 times the viscosity of water, pointing to the development of huge asymmetrically shaped micelles throughout the bulk of sol.

5, *h*. On inquiry into sedimentation velocities and sedimentvolumes of eumyosin flocculums at various pH's, it has been noticed that they attain to a second optimum at pH 8.5, and, in certain circumstances, further to a third optimum at a pH about 3.

5, *i*. The sedimentvolume of eumyosin concentrated by a strong centrifugal force increases abruptly at a pH above 7. This means that the net charge on the protein molecules increases suddenly at this pH value so that the protein forms markedly elongated fibrils necessarily suited for clotting into a framework. Hence, the formation of a second minimum at pH 8.5 has been ascribed to the syneresis of the gelatinous deposit mass, which is a phenomenon characteristic to the framework structure of jelly.

5, *j*. It has been experienced that eumyosin is modified in solubility upon heating, becoming apparently either more soluble or less soluble according to circumstances.

When its precipitate is heated in plain water, both its genuine ability of salting-in and its innate precipitability in plain water are lost the more completely, the more strongly is the protein subjected to heat-action.

If the protein is heated at neutrality in the presence of salt, *e.g.*, potassium phosphate buffer, it is converted more or less entirely into a mixture of two distinct fractions, namely, a heat-incoagulable portion and a portion incapable of salting-in. In extremely diluted solutions of the salt the protein becomes primarily the heat-incoagulable matter, while it becomes chiefly incapable of salting-in if heated at excessively high salt concentrations.

When eumyosin is heated in acid-or alkali-solutions, while it suffers no appreciable change in solubility as far as the reaction is close by the isoionic point, it turns as a rule to a pronouncedly soluble matter if the reaction is remote from the isoionic point. It has been observed in the latter case several phenomena seeming to point to the disintegration of the protein micelles into finer particles.

5, *k*. Citing an instance, it has been pointed out that the gelation of eumyosin may certainly be such a phenomenon as is first realizable only with a concentrated system consisting of moderately hydrophilized fibrous particles. Since this phenomenon of spontaneous setting of eumyosin deposit into an elastic mass which has been proved to be essentially equal to that of normal *Kamaboko*-jelly occurs most prominently in a peculiar zone of pH, *e.g.*, around 8.5, it has been termed protonogelation. Protonogelation is a characteristic behavior of intact eumyosin and seems based upon the same colloidal principle as its *Kamaboko*-forming ability.

It has been pointed out experimentally that the extent to which the protonogelatability is damaged on heating is only small at a pH whereat the protein is little soluble but is large at a pH inducing high solubility of eumyosin. Any quantitative correlation, however, remains as yet uncertain, due to complex effect of hysteresis.

5, *l*. It has been ascertained by experiment that spontaneous- or thermal-coagulation observed upon concentrated suspension or deposit of eumyosin is cyclically reversible at least so far as the solubility and heat-coagulability in NaCl-solutions are concerned. The spontaneous coagulation brought about by concentration advances to the higher extent, the higher the extent and the temperature of concentration. It proceeds most slowly at the pH of isoionic reaction. At the isoionic point, though the eradication of net charge favors the protein particles to be aggregated into flocculums so large that they settle promptly, the development of total charge prevents the particles from mutual adhesion so intimate as to yield unresolvable coagulums. The state of affairs gives rise to a problem that at least with the case of eumyosin *flocculation and coagulation are the phenomena to be kept distinct from one another* as regards the nature of origination.

6. On the basis of the experimental results obtained in the present work, also referring to the foreign works available, an inference has been drawn about the features and behaviors of molecules, micelles, and their aggregates of eu-

myosin, from which it has been arrived at a consistent interpretation for the colloidal phenomena related to *Kamaboko*-forming ability of eumyosin, *e.g.*, flocculation, peptization, gelation, syneresis, heat-coagulation, thermal disintegration, *etc.* In this case four factors have been taken into consideration: (1) The influence of intermolecular or intramolecular forces of attraction for which LONDON-VAN DER WAALS forces are always responsible whose effect, in turn, varies with the casual feature of the protein particles under consideration, while also the COULOMB forces between the reciprocally opposite charges belonging to different particles may often contribute to them with the case of protein. (2) The influence of intermolecular or intramolecular repulsive forces which are primarily traceable to the net charge depending upon the electrolytic dissociation of ionogenic groups on the protein molecules. (3) The influence of thermal agitation which is variable in response to the gross size as well as to the flexibility or segmental mobility of the fibrous particles. (4) The influence of the external stress applied to or the degree of packing, *i.e.*, the concentration of protein particles in the system.

Main subjects dealt with in this connection are arranged as follows:

6, *a*. Discussing the reaction mechanism by which fibrous particles of eumyosin become linked together into a framework building-up the bulk of jelly, it has been explained the reason why the protein undergoes gelation when made hydrophilic to an intermediate extent. Thus a theory has been given about the fundamental phenomenon for *Kamaboko*-jelly formation wherein eumyosin deposit does not remain liquefied in the presence of a weak peptizing agent such as NaCl or highly diluted NaOH but solidifies spontaneously into an elastic mass of jelly. It has been assumed that in the process of gelation, only some specially activated segments of interacting fibrils come into contact with one another getting over a potential barrier, leaving the other segments behind as they remain substantially hydrated.

The formation of a second minimum in the sedimentvolume of eumyosin at a pH distinct from that of the isoelectric point has been attributed to syneresis occurring in a gelatinized mass of deposit, *i.e.*, a framework of fibrous particles aggregated in alignment in some regions but amorphously in other regions.

6, *b*. The phenomena of heat-coagulation and thermal denaturation of eumyosin have been elucidated from a colloid sciential point of view, after which it has been concluded that *Kamaboko*-forming ability of eumyosin depends entirely on that whether the protein particles are secured or not in regard to their innate micellar construction.

6, *c*. Every stage of *Kamaboko*-manufacturing process has been given interpretation in the light of colloid chemistry of myosin.

The main reaction underlying the formation of *Kamakoko*-jelly has been concluded to be a colloidal phenomena as follows which is given rise to between virtually hydrophilized gigantic fibrous particles: Within the raw material fish flesh crushed, washed, and strained beforehand, there prevails salt-bridge among myosin particles, which prevents long

range polar VAN DER WAALS-LONDON forces exerted by peptide linkages arranged throughout but in the depth of the protein molecules from bringing the particles into close contact with one another. On the addition of NaCl to the strained flesh, however, the salt-bridge is substantially loosened ; and only a small number of segments of the fibrous particles, which have been specially activated by chance become agglutinated among different fibrils by the VAN DER WAALS-LONDON forces, resulting in a network of elastic jelly.

7. It has been investigated the tenability of dry eumyosin :

7, *a*. Carrying out preliminary experiments, a procedure has been settled by which eumyosin recoverable from its dried preparation is determined quantitatively.

7, *b*. It has been ascertained that the disappearance of recoverable eumyosin, which proceeds spontaneously during dry storage, originates in two distinct phenomena, namely, coagulation into a difficultly-peptizable matter and disintegration into a hardly-precipitable, heat-incoagulable substance.

7, *c*. It has been found that eumyosin becomes difficult to be recovered the more readily, the higher the humidity of storage, and hence the larger the moisture content of the stored material. It has also been made clear that the loss in peptizability of stored dry eumyosin is restricted within a finite degree.

7, *d*. It has been revealed that eumyosin powder does not undergo any measurable increase or decrease in the content of anhydrous matter even if it is moistened and becomes reddish yellow. It is conceivable that the moisture absorbed in the bulk of protein mass plays a role of lubricant favoring the mutual packing of protein particles but does not enter into combination with eumyosin.

Considered from various angles, the appearance of orangish color accompanying the loss in peptizability seems partly traceable also to the development of such a special disposition of protein particles, especially of their anoionic groups, as gives rise to an opitical structure-sensitive property throughout the protein mass.

7, *e*. To verify the inference that the spontaneous loss in peptizability of eumyosin during dry storage may be caused by the development of oriented or crystalline regions within the protein mass, powder photographs of *X*-ray diffraction have been taken, the microphotometer tracing of which confirms the inference to be valid.

7, *f*. About the nature of the sigmoidal course along which the phenomenon of difficultly-peptizable-becoming, DPB in abbreviation, of eumyosin was found to proceed during dry storage of the protein, some detailed discussions have been put forward in the light of modern protein chemistry and colloid science. Thus, the origin of the occurrence of DPB has been attributed to intermolecular and intermicellar replacement of salt-bridge by hydrogen-bonding in regard to their prevalence.

8. On the basis of the experimental data including those described in the

papers dealing with the present studies, it has early (Feb. 18, 1941) been applied to the Patent Bureau of Japan for a patent right "The simple procedure for the preparation of such dried fish as long remains available for *Kamaboko*-manufacture." The procedure has been registered as Japanese Patent No. 158070 in 1943. The main point of the procedure consists in: raw fish is first brought to the isoionic point of eumyosin, then strained from excessive water by pressing, and dried up finally at a low temperature.

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Errata

Most serious errors or misprints left uncorrected in the previous paper, Part II (HIRANO, 1953), are given in the following (see also foot note 45 in the present paper):

Place	*Erroneous*	*Correct*
Page 127, Table 15, b	Temp of measurement=60° C	Temp. of measurement=25° C
Page 135, Fig. 12	Curve 1′	Curve 2
" "	Curve 2	Curve 1′
Page 154, Line 17	100 *cc*	1000 *cc*
" Line 21	(500 *cc*)	(750 *cc*)

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ON THE ENERGY NECESSARY FOR BREAKING THE NETTING CORD BY SINGLE BLOW

Tadashi NAGATA

(Received May 20, 1953)

According to Prof. M. TAUTI,[1] when the cotton netting cord is subjected to the repeated blow, the greater the velocity and the mass of falling weight which inflicts the blow, the less energy consumed by the cord until it breaks. In the case of a single blow, F. SHIMIZU[2] has ascertained that the energy necessary for breaking is almost constant independently of the velocity as well as of the mass of the impact, while K. HONDA,[3] utilizing the same apparatus of Shimizu, has shown that the necessary energy increases as the velocity or the mass of the impact is increased.

In these investigations, in which the velocities of blows being less than 3 m/sec, the effect of the velocity or the mass of the impact on the breaking energy seems inconsistent with each other.

In connection with this problem, the present writer repeated the experiments, extending the range of the impact velocity to about 100 m/sec, and it has been ascertained that the energy necessary for breaking the cotton netting cord by a single blow is almost constant with no relatation to both the impact velocity and the weight. Two sorts of apparatus were used, one "*The high speed apparatus,*" and the other "*The low speed apparatus.*"

The High Speed Apparatus

Fig. 1 shows the out-line of this apparatus. B is a small rigid projection mounted on the circumference of a heavy rotating wheel which is driven by a variable speed motor M.

A small piece of steel rod A fixed to the one end of the test cord is kept just above the orbit of the small projection in the vertical line through the center of the wheel by means of an electro-magnet E, while the other end of the cord is tied to a hook on a ballistic pendulum P_1. When the circuit of the electro-magnet is opened, the rod A is struck by B, flying horizontally with the velocity v_0. Then the pendulum P_1 holding the other end of the cord is pulled ahead, while, as the period of free oscillation T_1 of this pendulum is chosen sufficiently large, it has no time to follow the sudden movement of the rod A. Namely, the rear end of the cord fixed to P_1 remains for some short duration at its position of rest as if it were tied to a fixed body, resulting in the rapid extention of the cord until its breakage while the velocity of the rod is diminished. If the velocity of the rod is v at the instant of the breakage of the cord, the total momentum communicated to P_1 should be equal to the decrease in that of the rod, so we get

$$M_1 V_1 = m(v_0 - v) ,$$

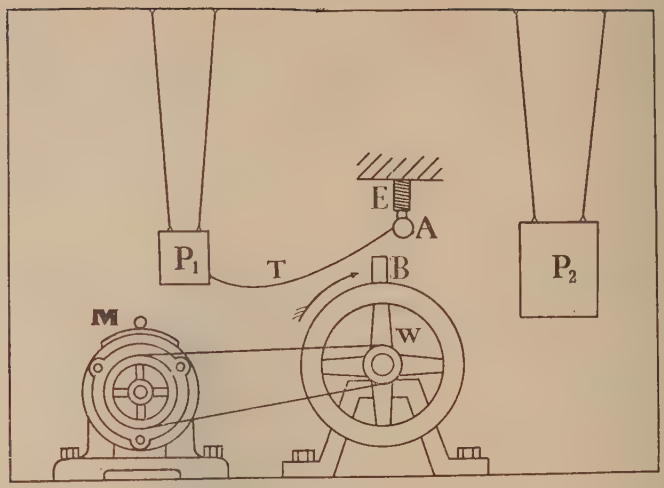

Fig. 1. *"The high speed apparatus."*

where, M_1 being the mass of P_1; V_1, the starting velocity of P_1; m, the mass of the rod. As for V_1, we can estimate from the amplitude of the first swing of the pendulum (α_1), by the following relation

$$V_1=\frac{2\pi}{T_1}\alpha_1 .$$

Thus we get

$$v_0=v+\frac{M_1}{m}\frac{2\pi}{T_1}\alpha_1 . \qquad (1)$$

After the breakage of the cord, the rod is assumed to run into the second pendulum P_2 with the velocity v, neglecting the air resistance with the short duration of free fleight, and P_2 is forced to swing with the first amplitude α_2. Then v can be determined by

$$v=\frac{M_2+m}{m}\frac{2\pi}{T_2}\alpha_2 , \qquad (2)$$

where M_2 is the mass of P_2, T_2 is the natural period of P_2. Therefore, we can estimate the energy E necessary for breaking the coad by

$$E=\tfrac{1}{2}m(v_0^2-v^2) . \qquad (3)$$

The angular velocity of the wheel 14″ in diameter was controlled from about 600 to more than 3000 r. p. m. The rod is not so heavy, that its kinetic energy is not large enough to cut the cord when its velocity is less than about 15 m/sec. For the low speed test with heavier weight, it is required to employ the different type of apparatus.

The Low Speed Apparatus

In the repeated blow test by Dr. TAUTI, if the cord is cut by the n-th

falling of the weight of mass m, from the height h, the energy E required for breaking is nearly $E=mgh$. When the cord, however, cut by the first falling of the weight, it is only said that E is less than mgh.

Thus the present author has designed an apparatus that enables us to get the energy necessary for breaking the cord by a single falling of weight. The principle is as follows: as shown in Fig. 2, the one end of the testing cord l in length is fixed to a bob M which is hung down by a spring, and the other end of the cord is tied to a weight m. If this weight m is dropped from a point A′, it posseses the velocity v_0 expressed by

$$v_0=\sqrt{2gh}\,, \qquad (4)$$

at the instant it passes through the point A, and the bob begins to be pulled downwards. If the period of the vertical oscillation of the system composed of the bob and the spring is fairly large, the cord will be broken off before the bob M is appreciably moved from its initial position. Thus the energy E for breaking the cord can be obtained from its amplitude of oscillation, which is recorded on the paper R hold vertically by a suitable indicater attached to the bob. In order that the bob may work ballistically, a weak spring is preferable, while some serious disadvantage arises that a weak spring may be streched beyond its elastic limit even by hanging statically a bob of considerable weight. Besides that, it is difficult to expect that the bob falls in the direction parallel to the vertical line so that the tip of the needle (a) may always keep contact to the recording paper R.

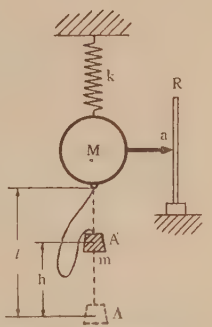

Fig. 2. Schematic diagram showing the principle of the low speed test.

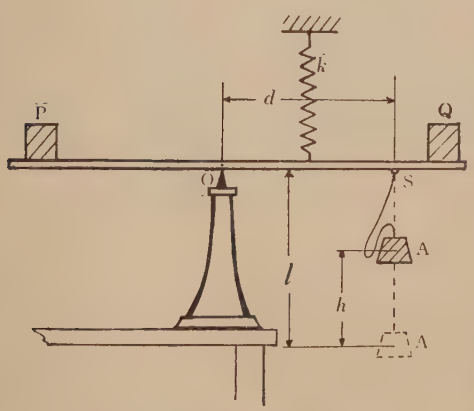

Fig. 3. *"The low speed apparatus"*

To remove these inconvenience, some modifications are made for the apparatus, as shown in Fig. 3. A rigid arm is made to turn freely around its midelle point O in the vertical plane including it, supporting two bobs P, Q at each end respectively, Q being a little heavier than P, while the slight unballance of the arm is canceled by a weak spring k. The arm starts to oscillate with the angular velocity ω after the cord has been broken, and since the angular momentum communicated to the arm must be equal to the change in the moment of momentum of the weight which is connected to the point S through the testting cord, it follows

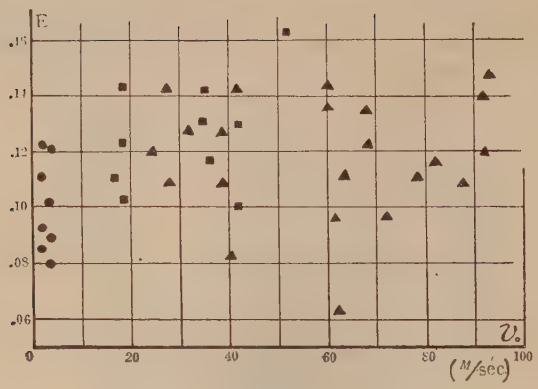

Fig. 4. The relation between the breaking energy in kg. m. of the 2-20's cotton cord 55 cm in length and both the velocity and the mass of impact. ▲; m=7 grs.: ■; m=23grs.: ●; m=1.3 kg.

$$I\omega = dm(v_0 - v)\,,$$

or

$$v = v_0 - \frac{2\pi}{T}\frac{I}{dm}\theta\,, \qquad (5)$$

where I being the moment of inertia of the arm; θ, the angular amplitude of oscillation of the arm Thus we get the energy E by

$$E = \tfrac{1}{2}m(v_0^2 - v^2)\,,$$

as in the case of the high speed test. The sensitivity can be adjusted by changing the distance d.

Result of the Experiment

The result attained for the 2-20's cotton cord 55 cm long is shown in Fig. 4. From the general trend, although there exists some inevitable scattering of data which seems to be attributed to the fundamental properties of the phenomenon of breaking or fracture, it may be concluded that mean energy necessary for breaking the cord has almost no relation to v_0 nor m.

In Fig. 5, the relation between the number of yarns of the cord (n) and the mean energy is shown. It is noticeable that E has not liner relation to n, but it increases more rapidly than the increase of thickness. This is remarkable inconnection with the fact that the breaking strength of the cord does not increase so much as the increase of the number of yarns.

Fig. 6 shows the relation between the mean breaking energy and the length of the cord of the same size. It is also interesting that E does not increase proportionaly to the length. When the netting cord is stretched with considerably high speed, it is often observed that the breakage occurs simul-

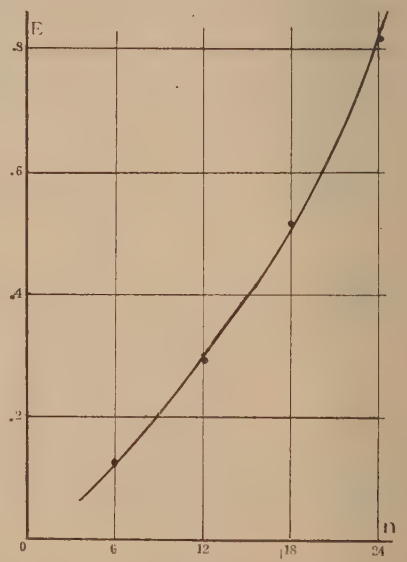

Fig. 5. The relation between the breaking energy in kg. m. of the 20's cord 55 cm long and the number of yarns n compsing the cord.

taneously at two points, and the cord is separated into three pieces. By adding lengths of all pieces, it is recognized that a permanent elongation has taken place. These elongations of cords after breakage tend to increase as the impact velocity decreases. It has been also ascertained that the number of cutting points has almost no influence on the breaking energy.

In a series of experiments above mentioned, the position at which the breakage occurs is not confined to any definite region, but is statistically distributed at random along the whole length of the cord.

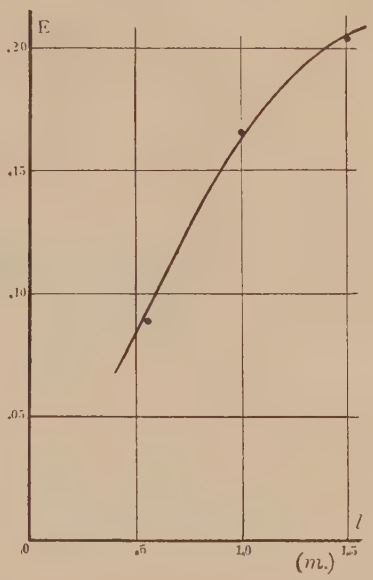

Fig. 6. The relation between the breaking energy in kg. m. of the 2-20's cotton cord and the length of the cord in meter.

In conclusion, the author expresses his best thanks to Prof. M. Tauti for his valuable advice, and also to Prof. M. Hirata of Tokyo University whose encouragement has been inspiring. He is also grateful to M. Ishigaki and N. Hirayama for their helps in the course of this experiment. This study was supported by the Scientific Research Expenditure of the Ministry of Education.

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ON THE FATIGUE OF NETTING CORDS UNDER CYCLIC LOADS

Katsuji Honda

(Received May 30, 1953)

The main purpose of this paper is to treat the problem of the some fatigue properties of netting cords subjected to the cyclic loadings. Making use of the apparatus deviced by J. Shioiri[1] with the object of investigating the dynamic elasticity of twines, the experiment required was practised. This apparatus is concisely illustrated in Fig. 1. The test cord, after having been kept under the

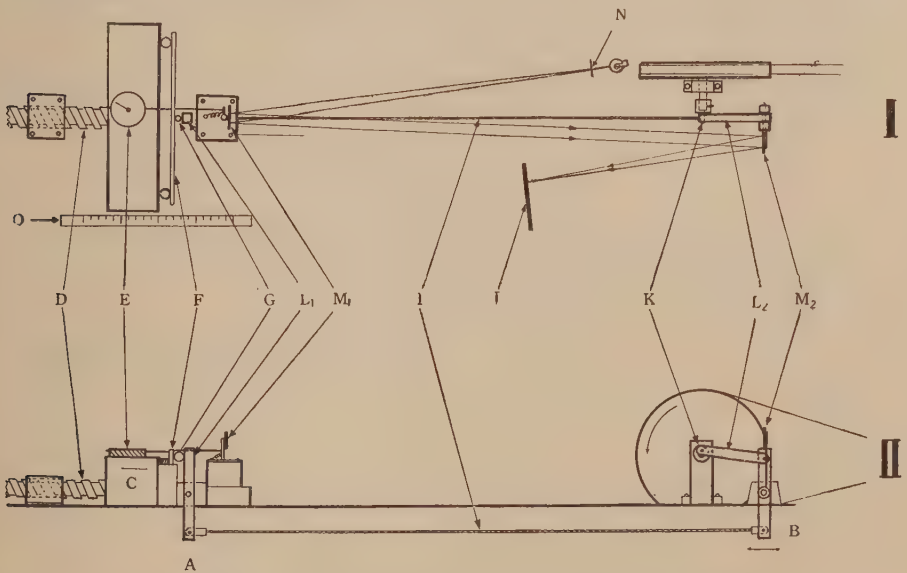

Fig. 1. Schematic diagram of apparatus for photographing the hysteresis loop of the cords.

I. Plane figure	II. Side view	A; B. Chuck
C. Bed	D. Screw bar	E. Dial guage
F. Steel bar	G. Steel ball	L_1; L_2. Lever
M_1; M_2 Mirror	I. Cord sample	J. Printing paper
K. Eccentric shaft	N. Pin hall	O. Scale

circumstances of a preparatory static load for several hours before the test, is fixed by its ends respectively to the chucks A and B and is stretched to a certain extent with any initial tension given. Then the lever L_2 with chuck B is forced to swing at the rate of 365 cycles per minute by a crank driven by a moter, and hence the tension to the cord as well as the length of the cord are repeatedly increased or decreased. By the above device, the lever L_1 with chuck A under

the influence of the tension in the cord bends the steel bar F through the small steel ball G. The deflection of the bar indicated by a dial guage E represents the value of the tension in the cord and the mirror M_1 which is setted to observe the angle corresponding to this deflection is made to turn around a vertical axis, while the other mirror M_2 is made to turn around a horizontal axis with the object of observing the angle corresponding to the elongation of the cord. An image of an illustrated pin hole N is formed on the screen being reflected by the mirror M_1 and M_2, thus appling the principle of optical lever, while the behaviour of the image on the screen indicates the stress-strain curve or the hysteresis loop.

To compensate the decrease in the initial tension of the cord due to its stretching during the test the bed C with lever L_1 is gradually moved backwards. (to the left side in Fig. 1) The hysteresis loop on the screen is photographed every about one hour, and at the same time the elongation of the cord is measured by the displacement of the bed C adjusted.

In the present case, the physical properties of the treated netting cord and the initial tension applied are shown in Tab. 1.

Tab. 1. Physical properties of the netting cords tested and the initial tension applied to the cords.

(a) The first figure represents size of single yarns (denier (D) for Amilan and Kurehalon, and yarn number for Cotton Kanebian and Kuremona); The second number of plies; the last character "Z" right handed twist.
(b) Size of manila hemp cord represents circuit in m.m.

Kind of cord	Construction	Twist per foot		Tensile strength Dry (kg)	Initial tension (kg)
		Upper	Lower		
Cotton	a) 20/8 3/60 "Z"	40	98	33	15
Kanebian (Vinylon)	〃 〃 〃	36	85	27	13; 13.5; 18
Kuremona (〃)	〃 3/90 〃	38	87	26	10; 16
Amilan	250D 3/60 〃	30	40	36	17
Kurehalon	300~400D 3/75 〃	30	51	60	25
Manila twine	b) 9 mm 〃	25	25	82	50

In Plate V, surface appearance of the cords subjected to cyclic loads is shown. Formerly, WALLER and ROSEVEARE[2] have pointed out that a large number of filaments broken are seen along cords in giving cyclic stresses to the cotton and rayon cords. As shown in Plate broken fibers of cord surface are observed along the cord looking nappy. The longer the cord is subjected to cyclic loads, the more the fibers are broken. In a tensile test, broken fibers are scarcely observed along the broken cord differing from the result in this fatigue test. But it is generally impossible to watch the crack growth in an individual fiber. Consequently, the observation mentioned above suggests the following discussion: The individual fiber is broken with the repeated cycles continued for several hours and in such a case the treated of cord is reduced in strength in comparision with that of untreated one, and finally the fatigue failure of the

cord is effected from the fact that the number of fibers being broken become so great in some locus that the fibers remaining unbroken may be also ruptured because of suffering the increased load imposed per fiber. The typical hysteresis loops obtained by the above mentioned test for various cords are shown in Fig. 2. There are some differences in the type of loop depending upon the kind of materials for the cords. These differences may be due to properties of twisted cords constructed of different materials respectively.

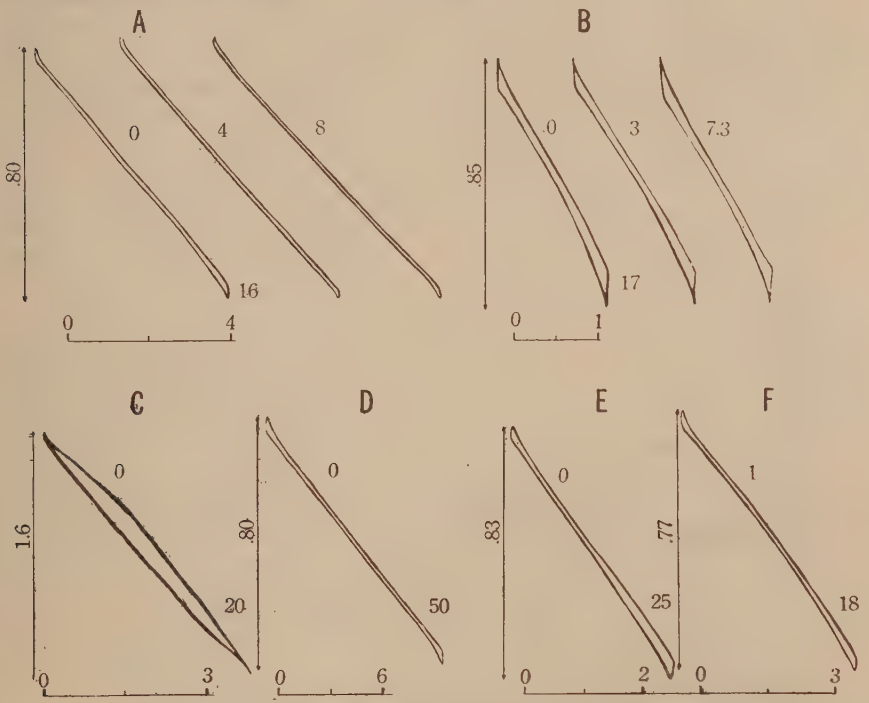

Fig. 2. Photograph of hysteresis loops for the cords.

There are photographed after giving the cyclic loading for several hours. Upper numerals associated with the loops show the hour and lower ones initial tension (Kg). Also showing the relation between stress (Kg) and strain (%) (ordinate) on the hysteresis loops.

A. Kuremona 20/8 3/60
B. Amilan 250 D 3/60
C. Cotton 20/8 3/90
D. Manila twine 9 mm
E. Kurehalon 300-400 D 3/75
F. Kanebian 20/8 3/60

The area within a loop represents the energy loss absorbed by the cord during one cycle of the cyclic loading. It seems that the above area tends to decrease with the practice of the repeated cyclic loading for several hours. The energy loss absorbed by the cord in the interval of two successive photographing of these loops is caluculated approximately by multipliyng the number of cycles performed in this interval by the arithmetic mean of the respective areas within the loops photographed at the beginning and the end of this interval.

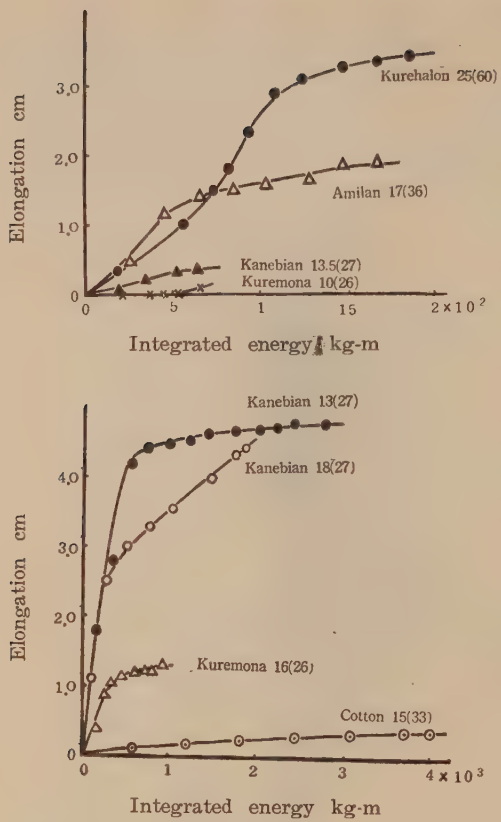

Fig. 3. Relation between integrated energy and elongation.

Numberals given in brackets show values of tensile strength (*kg*); the ones outside brackets initial tension (*kg*).

The relation between the integrated energy loss E_1 absorbed by the cord up to the n-th cycle and the elongation of the cord measured at the same time is shown in Fig. 3.

The energy loss absorbed by the cord during cyclic loading may perhaps be dissipated by the stretching of cord, by the frictional heat produced in the interior of the cord, and by the failure of fiber.

Energy E_2 neccessary for stretching the cord up to the n-th cycle is approximately computed by multiplying the initial tension by the elongation of cord at the n-th cycle.

Then the energy E_1 and E_2 are calculated by the twisted cords

Tab. 2. This table shows the values of $E_2/E_1(\%)$.

Cord	Initial tension (*Kg*)	E_2/E_1 Integrated energy (*kg-m*)		
		80	1,000	1,800
Kanebian	13.0	.29	.58	.34
〃	13.5	.01		
〃	18.0	.42	.63	.42

constructed of the same kinds of fiber. In Tab. 2, the values of E_2/E_1 are shown to those of some integrated energies, e.g., 80, 1,000, 1,800 *kg-m*. As shown in Tab. 2 values of E_2/E_1 are very small. Consequently the main portion of the energy consumption is not attributable to the stretching of cord but by the another work mentioned above. And also initial tension applied to the cords is almost independent of the values of E_2/E_1.

Therefore, more essential elements of the energy loss due to cyclic loading will be treated in the near future.

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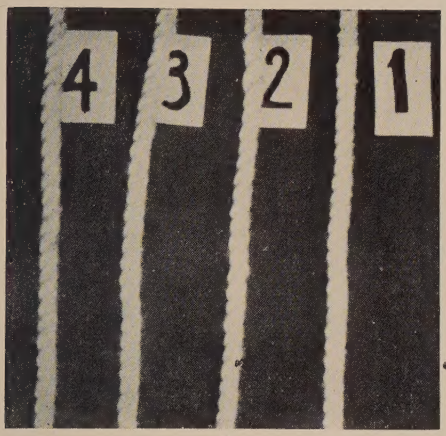
PLATE V

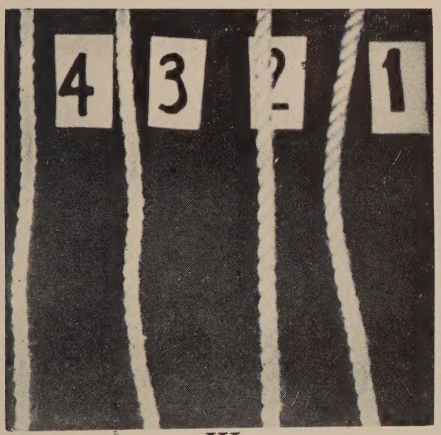
The photograph of the cord surfaces.

No. of photograph	Numeral given in photographs	Kind of cord	Initial tension (kg)	Applied hour of cyclic lording
I	1	Cotton 20's 3/60	—	Untreated
	2	〃 〃	15	6.5
	3	〃 〃	21	.8
	4	〃 〃	20	6.3
II	1	Kanebian 20's 3/60	—	Untreated
	2	〃 〃	13.5	5
	3	〃 〃	18	8.5
	4	〃 〃	13	13.3
III	1	Kuremona 20's 3/90	—	Untreated
	2	〃 20's 3/60	10	5.3
	3	〃 〃	—	Untreated
	4	〃 〃	17	8
IV	1	Manila twine 9 mm	—	Untreated
	2	〃 〃	50	1.8
	3	〃 〃	〃	.1
V	1	Amilan 250 D 3/60	—	Untreated
	2	〃 〃	17	8.3
VI	1	Kurehalon 300～400D 3/75	—	Untreated
	2	〃 〃	25	10.7



I

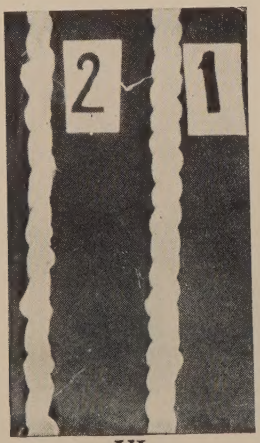
II



III

IV

V



VI